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EDITORIAL

THIS Journal was founded twenty-six years ago by the late E. A. Bunyard, F.L.S., whose particular interest was in systematic or descriptive Pomology. During the early years of the development of our Horticultural Research Stations in England, emphasis on this aspect was essential; for the investigator must first be able to identify and become intimately acquainted with his experimental material.

In 1923, the Long Ashton and East Malling Research Stations, both mainly concerned with fruit research, became partners in, and financially responsible for, the Journal, which they adopted as their official medium of publication. They invited other horticultural research institutions—The Cambridge Horticultural Research Station, the Cheshunt Experimental Station and the John Innes Horticultural Institution—to contribute papers by members of their staffs and thus help the Journal to cover a wider field of horticultural science. Its present title, *The Journal of Pomology and Horticultural Science*, was then adopted.

The word Pomology has never been a really satisfactory one to describe the activities of even the horticultural scientist devoting his attention to the different kinds of fruits and their reactions to various cultural and environmental factors. The term Horticultural Science, on the other hand, describes much more accurately the ever enlarging field of horticultural investigation—including such widely differing subjects as the genetics, breeding and classification of horticultural crops, the choice and maintenance of soils for horticultural purposes, problems of growth and fruitfulness in relation to chemistry, plant physiology and the principles and practices of management, the protection of fruit crops from diseases and pests, and the quality, storage relations and effective utilization of horticultural produce.

Investigations into the potential life of and conditions for storing, and even processing, fruits and vegetables have been greatly intensified during the past two decades, and the Journal has, for many years, welcomed contributions from the Low Temperature Research Station of the Department of Scientific and Industrial Research on these subjects. Finally, there is every prospect of the development of increased research activities on commercial vegetable and ornamental flower cultivation, while the establishment of associate research and experiment stations, to include horticultural projects, is envisaged.

It is thus apparent that in the course of a few years the output of papers on all these closely related aspects of Horticultural Science will make it desirable officially to recognize the ever widening scope of our Journal by dropping the redundant word Pomology from its title, calling it simply *The Journal of Horticultural Science*. This shortened and more convenient title will therefore be adopted for Volume XXIV and succeeding issues.

Owing to the abnormal conditions prevailing during the past eight years, it has unfortunately been impossible, as formerly, to ensure the issue of a Volume, of four separate parts, within each calendar year. Indeed, from 1939 to 1946, inclusive, it has been possible to complete Volumes XIX to XXII only at longer and irregular intervals. This, of course, has been due not only to the shortage of paper and of skilled printers, but also to the temporary deflection of the activities of many horticultural research workers into various pressing short-term practical projects—and

this has meant a reduced and irregular submission of manuscripts dealing with the results of longer term investigations on more fundamental problems.

However, the present prospect of completing Volume XXIII in the current year is more hopeful, and the outlook for the future much more promising. Meanwhile, we wish to thank those contributors and subscribers who have made it possible for us to maintain the Journal at all through the long periods of crisis. We also wish to pay tribute to the assistance given us by our Publication Committee, our Assistant Editor and Associate Editors, as well as to Messrs. Headley Brothers, our printers and publishers.

We much regret that owing to the general heavy increase in cost of production, it is inevitable that the price of the Journal must be increased. The subscription therefore will be raised from 25s. to 35s. per Volume of four parts (10s. per separate part) as from the issue of the first part of Volume XXIV, in 1948. The Journal is not the organ of, nor is it subsidized by, any " Association of Horticultural Scientists ", or other such body ; it has to pay its own way on its merits. We confidently hope that it will continue to give satisfaction to our subscribers and we trust that we may still count on their valuable interest and support.

R.G.H.
T.W.

May, 1947.

LABORATORY STUDIES ON THE TOXICITY OF HYDROCARBON OILS AND SIMILAR SUBSTANCES TO THE EGGS OF SOME COMMON ORCHARD PESTS

I. GENERAL INTRODUCTION

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Most of the large amount of published research on the ovicidal action of hydrocarbon oils has consisted of determining certain more or less arbitrarily chosen physical and chemical properties of commercial washes or, less frequently, of wide oil fractions, and attempting to correlate them with the observed toxicity. Only in a few instances has any attempt been made to examine constituent fractions of the oils. The earlier work has been fully reviewed by Martin (1935) and only general conclusions need be mentioned here. The more significant results will be discussed in greater detail in subsequent parts of this series of papers describing tests with particular organisms.

It was known from field experience that the commoner insect pests controlled in the winter egg stage fall into two groups distinguished by their reaction to treatment with petroleum oils and tar oils respectively. Austin, Jary and Martin (1934) made a detailed comparison of the toxicity of petroleum and high temperature tar distillates to eggs of *Lygus pabulinus* L. and demonstrated the superiority of the former type. They suggested that the nature of the hydrocarbons present in the oils had a significant effect on toxic action, but they were unable to establish any difference between petroleum oils of similar boiling range but different base, notably asphaltic, naphthenic, and paraffinic. They had previously shown (1932) that petroleum oils are markedly inferior to tar oils in toxicity to the eggs of Aphis and Apple Sucker. They also showed that cotton seed oil and mustard oil are highly toxic to *L. pabulinus*. Staniland, Tutin and Walton (1930) had put forward the hypothesis that two specific types of toxic action exist, viz. : the physical or stifling effect of petroleum oil, exhibited against eggs of capsids, some caterpillars, and Red Spider, and the chemical or direct poisonous action of tar oils against Aphis and Apple Sucker.

On the basis of these and other trials carried out by workers in several countries against a variety of test insects, tentative specifications for the two kinds of oil were put forward by Martin (1935). These, in modified versions but with the same essential basis, have since been adopted as official specifications in Great Britain. In them emphasis is laid on distillation range (volatility) of petroleum oils, and on the content of high-boiling neutral aromatic oil in the tar oils, as criteria of efficiency.

The present investigations began in 1934 in an attempt to evaluate low-temperature carbonization tar distillate as an ovicide. It was thought that this

material, being a by-product for which at that time there was little commercial outlet, might serve as the basis for a cheap tar-oil winter wash. Moreover, having affinities with petroleum oils by reason of its aliphatic constituents, it offered the possibility of combining the action of petroleum on Capsid and Red Spider eggs with that of tar oil on Aphis and Sucker eggs. Examination of these possibilities involved comparisons with the high-temperature tar oils and the petroleum oils commonly used in winter washes, and then developed into a survey of a wide range of hydrocarbon oils (including hydrogenation products) and an attempt to determine the factors involved in the ovicidal action of these oils.

Materials.

The Fuel Research Station, Greenwich, made possible a close standardization of some of the materials used by supplying distillates from low-temperature tar and horizontal-retort high-temperature tar of which the history and treatment were known through every stage, from the original coal to the final product. In this way variations between samples over several years were reduced to a minimum. The samples used from 1934 to 1941, inclusive, came from the same original bulk stocks of distillates. Similarly the Gas Light & Coke Company supplied samples of vertical-retort high-temperature tar distillates. Two hydrogenation products of coal origin were also used ; one, a hydrogenated low-temperature tar, was supplied by the Fuel Research Station and the other, a hydrogenated coal product, by Messrs. Imperial Chemical Industries, Limited. Other oils used were commercial grades of anthracene oil, creosote oil, fuel oil, spindle oil and kerosene, as well as special fractions and extracts of oils. Several of these last-named were supplied by Messrs. Technical Products, Limited. For the purpose of comparison a vegetable oil was included in a single test.

The following is a general description of the oils used ; the abbreviations inserted in brackets will serve to identify them in the subsequent papers.

Horizontal Retort High-Temperature Tar Distillate. (H.T.). Durham (Thornley) coal carbonized at *circa* 1,100° C. Yield 10 gallons of tar per ton of coal. Commercial cut to two fractions distilling respectively up to 250° C., and 250° C. to pitch.

Vertical Retort High-Temperature Tar Distillate. (V.T.). Yorkshire plus a little Durham coal carbonized at *circa* 1,350° C., with 10 per cent. steaming. Laboratory cut to two fractions, as above.

Vertical Retort Low-Temperature Tar Distillate. (L.T.). Barnsley (Dalton Main) coal carbonized at *circa* 650° C. Yield 18 gallons of tar per ton of coal. Commercial cut to two fractions, as above.

Hydrogenated Low-Temperature Tar. (Hydr. L.T.). Hydrogenated Dalton Main Low-temperature tar. Commercial cut distilling above 250° C.

Hydrogenated Coal. (Hydr. Coal). Residue above 300° C. from distilling the primary products of the hydrogenation of coal.

Special Heavy Tar Distillate. (Heavy Tar). A mixed distillate, 96 per cent. distilling above 300° C. and 35 per cent. above 400° C. Supplied by Messrs. Burt, Boulton and Haywood, Ltd.

Anthracene Oil (Anthr.). A commercial high-temperature carbonization product, distilling mainly between 250° C. and 400° C.

Creosote Oil (Creos.). Commercial samples of variable composition, distilling between 150° C. and 400° C.

Spindle Oil (Spin.). A semi-refined petroleum oil from Venezuelan crude, distilling substantially between 300° C. and 400° C.

Kerosene (Ker.). A refined, odourless, close-cut, petroleum fraction, distilling mainly between 200° C. and 250° C.

Fuel Oil (Fuel). A commercial light fuel oil, distilling mainly between 250° C. and 400° C.

Edeleanu Extract (Edel.). A sulphur dioxide-soluble fraction of petroleum obtained in refining a lubricating oil, distilling mainly between 300° C. and 400° C.

Furfural Extract (Furf.). A high-boiling (above 350° C.) petroleum extract, obtained in the solvent refining of a paraffinic crude oil.

Sulphur Oils. Egyptian petroleum oils containing 7-16 per cent. of combined sulphur, distilling between 150° C. and 350° C.

Cotton Seed Oil (C.S.O.). Commercial washed cotton oil. Supplied by the British Oil and Cake Mills, Ltd.

Details of the chemical and physical constants of these oils and of the fractions derived from them will be given in the separate parts describing the tests made with them.

The published literature on the composition of tar and petroleum oils, especially the fractions distilling above 250° C., is far from conclusive, but the following is believed to represent the concensus of current views (F.R.B., 1931).

The neutral oil of a horizontal retort high-temperature distillate (H.T.) is almost wholly aromatic as determined by solubility in dimethyl sulphate, but a vertical retort high-temperature distillate (V.T.) contains up to 30 per cent. of material insoluble in dimethyl sulphate, and probably largely naphthenic. The remaining 70 per cent. or so of aromatic is, however, of a type not greatly dissimilar from a horizontal retort distillate. The low temperature distillate (L.T.) also contains some 30 per cent. of aliphatic material, but with a greater proportion of paraffins, and the aromatic components have longer and more numerous side chains than occur in the two other tar oils. Hydrogenation of low temperature tar distillate, especially where the higher boiling fractions have been re-circulated in the process, gives rise to a mixture of aromatic and aliphatic compounds of which the latter are largely, and in the higher fractions almost entirely, naphthenes with paraffinic side chains up to five carbons in length (Hall and Cawley, 1937). Hydrogenated coal is different again. It contains a higher proportion of aromatic material, but the aliphatic portion is mainly paraffinic, not naphthenic as in hydrogenated low-temperature tar distillate.

Although little is known of the identity of the individual substances present in spindle oil fractions it appears to be accepted that they comprise aromatic compounds, naphthenes, and *iso*-paraffins, with only small amounts of olefins (Mikeska, 1936). The relative proportions of these main constituents, and especially of the naphthenes and paraffins, varies widely according to the source of the oil. In the Venezuelan spindle oil used the proportion of aromatic components was low and of paraffinic components high. The Edeleanu extract, while still predominantly aliphatic, contained a substantial amount of aromatics ; the furfural extract was a mixture of aromatic, naphthenic, and paraffinic hydrocarbons with the aromatics present in larger proportion than in the original stock.

With the necessary caution and reserve the constitution of the oils used can be summarized as follows, basing the assessment of aromatic content on solubility in dimethyl sulphate:

H.T. Almost wholly aromatic of relatively simple types.

V.T. Mainly aromatic similar to H.T., with some mainly naphthenic aliphatic types.

L.T. Mainly aromatic of heavily substituted type, with some largely paraffinic aliphatics.

Hydr. L.T. A mixture of aromatic, as in L.T., with a larger proportion of aliphatic, itself mainly naphthenic or mixed naphthenic-paraffinic.

Hydr. Coal. Mainly aromatic, with an aliphatic portion consisting mainly of paraffins.

Spin. Mainly paraffinic, with very little aromatic.

Edel. Mainly aliphatic, with a considerable amount of aromatic.

Furf. Mainly paraffinic, with some aromatic.

The complexity of the situation is thus evident. Not to mention individual components, the types of compounds involved include aromatic, naphthenic, paraffinic, olefinic, mixed aromatic-paraffinic, and mixed naphthenic-paraffinic molecules. Chemical methods alone are quite inadequate to differentiate between these and recourse must be had to physical and physico-chemical methods also.

Methods.

The first broad surveys of the ovicidal properties of the oils were carried out on the whole oils as received and on the separated neutral, phenolic, and basic components when phenols and bases were present in appreciable amounts. These three components were separated by the usual process of washing the oil, usually in solution in ether, with dilute sodium hydroxide solution and dilute hydrochloric acid in succession and recovering the phenols and bases by appropriate means. The neutral oil was isolated by distilling the ether solvent after drying over anhydrous sodium sulphate.

The neutral oils were further fractionated by distilling in 50° C. or 25° C. cuts. It was recognized that this procedure is open to criticism on several grounds, some of which are discussed below, but it appeared to be the most useful approach to the problem of locating toxic fractions. The greatest difficulty arose from the physical behaviour of the oils on fractionation. Many of the distilled tar fractions, particularly those derived from high-temperature carbonization oils, crystallized on cooling. To attempt to work with them in this two-phase condition presented serious difficulties in manipulation. On the other hand, to separate the liquid portion and use that alone meant that the fractions would no longer represent in the aggregate the unfractionated oil. The latter procedure was adopted on the ground that it is no more than an extension of the principle accepted in the preparation of the original commercial cuts. It is necessary only to keep in mind that the results obtained are characteristic of the one fraction of oil used in obtaining them. The distillates were well cooled, allowed to stand for at least three hours, and separated by vacuum filtration. All the solids were retained for examination if such a course should later be found desirable. Because of variations in the amounts of oils available and in the size of the fractions obtained it was not possible to standardize the distillations,

and the temperature limits of the fractions must be regarded as plus or minus a few degrees. In the fractions of higher distillation range there was probably some pyrolysis of the oil, but in no case was the distillation continued to a point at which this became serious. For certain special purposes fractions other than the 50° or 25° distillation cuts were prepared. These are described in the relevant sections.

According to the needs of particular experiments, and subject to the availability of sufficient material, the following characteristics of the neutral oils and fractions were determined:

Specific gravity. Determined by sp. gr. bottle or pycnometer, according to the amount available, at 60° F.

Viscosity. In only a few cases was there sufficient material for a determination in the standard Redwood viscometer. In consequence the appropriate Ostwald instrument was used and the result converted to Redwood No. 1 figures by substituting in the expression

$$T = \frac{V + \sqrt{V^2 + 4AB}}{2A}$$

derived from $V = AT - \frac{B}{T}$ (I.P.T. 1935, Serial L.O.8).

where V=Kinematic viscosity (Ostwald).
T=Viscosity in seconds (Redwood).
A and B are instrument constants.

Where direct comparisons could be made between the calculated figure and that determined in the Redwood No. 1 viscometer, agreement within 1 per cent. was found. All determinations were made at 70° F.

Distillation range. Determined by standard method (S.T.P.T.C. 1938, Serial C.O. 3-38). All the earlier determinations were made by volume, and although in the later stages of the work it was appreciated that a measure by weight might have been more valuable, the former basis was continued so as to give comparable values.

Unsulphonated residue.
Solubility in dimethyl sulphate. } Determined by the methods described by Martin (1935).

Unsulphonated residue, being the least informative characteristic, was determined only for a minority of the oils when ample amounts were available.

During the later stages of this work a physico-chemical method (devised by Vlugter, Waterman, and van Westen (1935)) for estimating the aromatic, naphthenic, and paraffinic structures present in hydrocarbon oils came into use in the petroleum industry and was utilized by Pearce, Chapman and Avens (1942). Unfortunately it came to the notice of the present authors too late to be applied to the oil samples at the time the ovicide tests were made. Although most of these samples are extant, the probability of changes (especially polymerization) having occurred is so high as to invalidate the results of determinations that could have been made subsequently.

Although the complete validity of the Waterman analysis is not accepted by all authorities, and in spite of difficulties that arise with mixed molecules, this procedure seems to offer the best approach to further investigation of the relation of toxicity to constitution.

In all but a few experiments the oils were made up in a "1 per cent. soap" emulsion. One ml. of redistilled oleic acid for every 100 ml. of wash to be made was thoroughly mixed with the oil to be emulsified, and the mixture was poured

into distilled water containing the equivalent amount of sodium hydroxide. Usually this procedure gave excellent, stable emulsions, but when, because of high viscosity or some other feature of the oil, this was not so, the emulsion was passed through a hand-operated emulsifier. Throughout these investigations concentrations are reported by volume.

Test Organisms.

The chief test insects used were the Vapourer Moth (*Orgyia antiqua* L.), the Winter Moth (*Operophtera brumata* L.) and the Green Apple Aphis (*Aphis pomi* De Geer), and it is proposed to report the work on each of these pests in a separate part of this series of papers. To a much smaller extent use was made of the Fruit-tree Red Spider (*Oligonychus ulmi* C. L. Koch) and the Apple Sucker (*Psylla mali* Schmidt). The methods employed in securing adequate stocks of insect material and in its manipulation were those described by Steer (1938).

ACKNOWLEDGMENTS.

The authors gratefully acknowledge the co-operation of the Fuel Research Station of the Department of Scientific and Industrial Research, of Messrs. Technical Products, Ltd., and of the other commercial firms mentioned in the text, all of whom have materially assisted in these investigations by supplying samples of oils and data relating thereto.

SUMMARY.

The materials, methods, and test organisms used in a series of investigations on the toxicity of oils to insect eggs are described. The broad chemical classification of these oils is discussed.

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LABORATORY STUDIES ON THE TOXICITY OF HYDROCARBON OILS AND SIMILAR SUBSTANCES TO THE EGGS OF SOME COMMON ORCHARD PESTS

II. EXPERIMENTS ON THE EGGS OF THE WINTER MOTH (*OPEROPHTERA BRUMATA* L.)

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INTRODUCTION.

Very little of the large amount of research on the ovicidal action of hydrocarbon oils has referred specifically to the action on eggs of the Winter Moth (*Operophtera brumata*). Among the earliest of the workers to do so was Tutin (1928) who used the " Small Winter Moth ". His chief conclusions were that

(*a*) a high-boiling neutral tar oil distilling 280°-360° C. was superior to a lower-boiling oil distilling 190°-280° C.

(*b*) the presence of tar phenols lowered the toxicity of the oil.

(*c*) the nature and source of the tar were immaterial.

No data, however, were presented in support of these conclusions. Later, Staniland, Tutin and Walton (1930) compared several hydrocarbon oils and arrived at similar conclusions. They advanced the hypothesis that the Winter Moth type of egg is killed by a stifling action and that the toxicity of an oil will, in consequence, mainly be determined by the distillation range.

Many other workers, using wide cuts of oils against a variety of test insects, have produced evidence supporting the view that the oils of higher distillation range are the more toxic. Melander, Spuler and Green (1925) found that against eggs of the Fruit Tree Leaf-roller (*Cacoecia argyrospila* Walker) distillation range was the dominant factor in the toxicity of petroleum oils, and that viscosity, specific gravity, and the base of the oil were of little importance. Similarly, against the capsid bug *Lygus pabulinus* L., Austin, Jary and Martin (1934) found no consistent relation between toxicity and specific gravity, viscosity, unsulphonated residue, or nature of base, in a series of petroleum oils each of which distilled in the range above 300° C. They found indications, however, that in passing from one type of oil to another the effect of distillation range can be outweighed by other characteristics. Thus, a petroleum faction of high aromatic content, namely an Edeleanu extract, appeared to be less toxic than a straight petroleum oil, though the results, based on a very small number of egg counts, were not conclusive. Their earlier work (1932) had shown tar oils to be inferior to petroleum oils in toxicity to capsid eggs. On this evidence they suggested that the chemical nature of the oil, e.g. the content of saturated hydrocarbons, may be an important factor in toxicity. More recently, Pearce, Chapman and Avens (1942), working with Fruit Tree Leaf-roller, showed that within a selected series of petroleum oils, the most highly paraffinic were the most toxic. Penny (1921) had already put forward a similar suggestion based on the observed toxicities of oils of paraffinic and asphaltic base. These latter authors, however, did not report the distillation ranges of their oils.

The aims of the present work were :

(i) to locate more precisely the regions of maximum toxicity,
(ii) to search for constituent substances of high toxicity,
(iii) to correlate toxicity of fractions with chemical and physical constants.

EXPERIMENTAL

Methods and Materials

Chemical.—The oils used included a series ranging from aromatic tar distillates to aliphatic petroleum oils, together with cotton-seed oil. They have been described in Part I of this series and the abbreviations now used are the ones set out there.

Biological.—The biological material used in this investigation was the egg of the Winter Moth (*Operophtera brumata*), whose caterpillar is among those of greatest economic importance on orchard fruit. This egg is well suited to laboratory methods of testing ovicides, but since its production is seasonal, all tests were necessarily carried out in the winter months, December to March. In order to avoid undue variation in the state of development of the eggs, the shoots were treated as far as possible not later than mid-February ; the precise dates appear in the Tables of results. All dosages are reported as the concentration of toxicant applied to the eggs. The relation of this to the dosage effectively deposited is not necessarily rectilinear and may differ from oil to oil. Attempts to elucidate this relationship were not successful. The code numbers in the Tables indicate the number of each test and the season in which it was carried out ; thus 15/38 denotes test 15 of the winter of 1938-39. By this device tests in different seasons can be distinguished and the appropriate control series can readily be identified.

The method of testing was essentially Method 1 for Winter Moth described by Steer (1938) and consisted, in outline, of dipping infested shoots into the prepared liquid and then, after a preliminary draining in the laboratory, storing them in an outdoor insectary. When hatching had finished the hatched and unhatched eggs on each shoot were counted with the aid of a binocular microscope.

Normally five shoots were used for each treatment and in most cases the results are presented as the mean percentage hatch per shoot, together with its standard error. This method has the disadvantage that the standard errors are calculated from dissimilar components, since the number of eggs on the several shoots may differ fairly widely. Fortunately the effect of this is towards hiding real differences rather than towards making spurious ones appear significant. The objections to other methods of presentation that were considered are, on balance, greater than those to the one outlined.

DISCUSSION OF RESULTS.

Preliminary surveys.—In 1936-37 wide fractions of a number of oils were investigated. H.T., V.T., L.T., Hydr. L.T., Hydr. Coal, Creos., and Spin. oils, and the neutral oils isolated from the H.T., V.T., and L.T. were tested at 4 per cent. and 2 per cent. concentrations. Table I shows the results of these tests together with those obtained with more precisely cut fractions of H.T., V.T., L.T., and Hydr. L.T. neutral oils and with other oils of special interest (Heavy Tar and Edel.) that became available later. Inverted commas in Tables I and II indicate nominal or approximate data.

TABLE I.

Toxicity of wide fractions of oils.

Test No.	Date of treat-ment.	Material.	Conc. % by vol.	Mean % hatch.	S.E.	Total no. of eggs.	Neutral oil % by weight.	Sp. gr.	U.S.R. %	Vis-cosity Red. I. at 70° F.	Insol. in Me_2SO_4 %	Distilling range. ° C. 10%	50%	80%	%> 300
												by volume.			
14/36	25.ii.37	H.T. ">250° C." neutral	4·0	6·6	4·1	352	97·7	1·084	0		0	242	306	354	53
			2·0	23·2	1·9	516									
14/36	do.	V.T. " >250° C." neutral	4·0	18·4	5·8	299	98·4	1·012	20		33	272	306	342	56
			2·0	35·0	8·0	331									
14/36	do.	L.T. " >250° C." neutral	4·0	48·4	9·4	479	98·4	0·994	18		34	245	291	332	44
			2·0	66·5	1·8	308									
14/36	do.	Spindle oil	4·0	1·3	0·1	461	100	0·901	72	180	96	347	368	389	100
			2·0	20·4	5·0	333									
15/36	26.ii.37	H.T. " >250° C." whole	4·0	27·6	6·5	138	84·6	1·034	4		0	224	273	330	38
			2·0	33·5	4·4	113									
15/36	do.	V.T. " >250° C." whole	4·0	17·2	3·6	144	88·0	1·010	17		26	270	306	344	55
			2·0	33·4	8·3	148									
15/36	do.	L.T. " >250° C." whole	4·0	27·2	4·0	155	72·1	0·986	14		30	234	286	327	43
			2·0	38·5	6·7	268									
15/36	do.	Hydr. L.T. ">250° C." whole	4·0	12·1	5·4	107	99·2	0·979	18		66	264	313	356	58
			2·0	43·8	3·8	207									
18/36	1.iii.37	Hydr. Coal ">300° C." whole	4·0	[0]	—	11	96·1	1·082			20				
			2·0	[50·0]	[22·4]	17									98
18/36	do.	Creosote oil whole	4·0	[94·5]	[5·5]	24	81·3	1·023							
			2·0	[90·0]	[6·7]	54									
6/38	3.ii.39	H.T. " >250° C." neutral	4·0	19·9	7·4	284	100			320	0				
			2·0	29·4	2·0	435									
6/38	do.	V.T. > ,, ,,	4·0	32·8	3·3	438	100	1·027		85	26				
			2·0	45·6	2·2	319									
6/38	do.	L.T. > ,, ,,	4·0	37·7	3·8	428	100	1·026		99	26				
			2·0	40·0	5·2	375									
6/38	do.	Hydr. L.T. > ,,	4·0	24·9	4·9	422	100	0·997		86	66				
			2·0	52·0	3·1										
14/36	25.ii.37	Control	—	92·8	1·4	367									
15/36	26.ii.37	,,	—	96·1	2·3	117									
18/36	1.iii.37	,,	—	[96·0]	[2·8]	52									
6/38	3.ii.39	,,	—	90·8	1·6	470									
8/38	7.ii.39	Heavy tar neutral	3·0	31·0	5·8	269	100								96
			1·5	37·4	5·9	300									
6/37	17.ii.38	Edeleanu extract	2·0	41·7	6·3	270	97·2	0·997	16	351	22	321	357	390	94
3/38	30.i.39	Cotton Seed Oil	3·0	3·6	1·2	324		0·921		343					
			1·5	13·0	3·7	120									
6/37	17.ii.38	Control	—	94·5	1·4	303									
3/38	30.i.39	,,	—	94·9	2·8	208									
8/38	7.ii.39	,,	—	89·7	2·0	507									

Not much importance can be attached to results based on the few eggs used in the tests of Hydr. Coal and Creos. oils. The remaining data of 1936-37 suggested that distillation range was the principal factor determining toxicity and, less obviously, that the content of neutral oil distilling above 300° C. might serve as a convenient index of toxicity to Winter Moth eggs. The mortality in probits was accordingly plotted (Fig. 1) against the logarithm of the dosage of neutral oil distilling above 300° C., ascertained from distillation data.

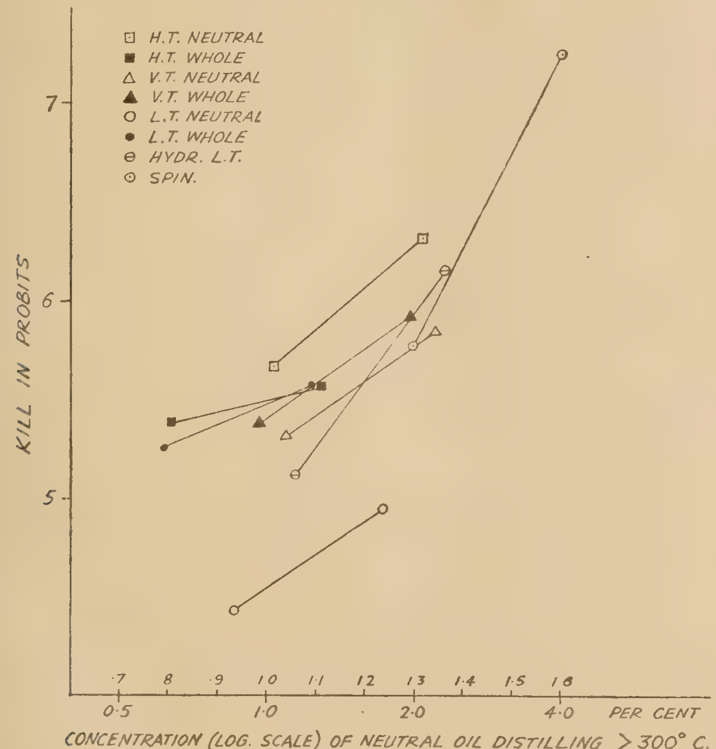

FIG. 1.

Log concentrations of neutral oils distilling above 300° C. plotted against mortality in probits.

Fair agreement is apparent, but the L.T. neutral oil is over-valued and the Spindle oil under-valued. The further evidence of the tests of Heavy Tar and Edel. (Table I, Tests 6/37 and 8/38) is less in accord with a hypothesis that the toxicity of an oil can be inferred from its content of a particular high boiling fraction.

From Fig. 1 it would appear that the dosage mortality line for the Spindle oil is much steeper than those for the other oils, all of which are roughly parallel. These curves, however, are based on two points only, and several subsequent determinations of regression coefficients from more adequate data, e.g. Fig. 4, did not substantiate the difference. It probably arose from a chance anomalous result for the 2 per cent. concentration.

Tests with the more precisely cut > 250° C. fractions of H.T., V.T., L.T., and Hydr. L.T. neutral oils (Table I, Test 6/38) suggested that the removal of lower boiling fractions decreased the discrepancy between the L.T. and the other oils. Further evidence supporting this view was obtained in a later experiment (page 17), where fractions of H.T. and L.T. neutral oils distilling between 320° C. and 380° C. did not differ significantly in toxicity.

It seemed that the marked superiority of the L.T. whole over the L.T. neutral oil might lie in the high content (19 per cent.) of phenols in the whole oil. Accordingly the phenols and bases obtained in isolating the H.T., V.T., and L.T. neutral oils were tested in 1937-38 with results that appear in Table II. It has already been shown (Shaw and Steer, 1939) that pure quinoline—a tar base—at 0·5 per cent. is only moderately toxic to Winter Moth eggs.

TABLE II.

Toxicity of tar phenols and bases.

Test No.: 2/37. Date of Treatment: 20.i.38.

Material.	Conc. % by vol.	Mean % hatch.	S.E.	Total No. of eggs.
H.T. " >250° C." phenols ..	1·0	52·4	8·9	253
,, ,, ,, ..	0·5	75·3	8·2	164
V.T. ,, ,, ..	1·0	79·4	6·3	194
L.T. ,, ,, ..	1·0	73·0	7·8	234
H.T. " >250° C." bases ..	0·2	79·7	5·2	238
V.T. ,, ,, ..	0·2	63·3	2·4	217
L.T. ,, ,, ..	0·2	69·7	6·4	252
Control	—	90·3	1·9	223

The concentrations used in these tests were at least equal to the amounts of phenols or bases present in the concentrations of whole oils shown in Table I. The results obtained do not support the view that a high content of phenols could account for the superiority of the L.T. whole over the L.T. neutral oil. But the possibility that these substances behave differently when dissolved in oil must not be overlooked. It has been shown (Hurst, 1940) that a polar substance, non-toxic when applied alone, can show a high toxicity to the larvae of certain insects when applied in solution in a non-polar, non-toxic solvent.

TESTS OF FRACTIONS OF NARROW DISTILLATION RANGE.

In 1937-38 and 1938-39 a systematic examination of toxicity was made over the whole distillation range of several widely different types of tar and petroleum oils. For this purpose the oils were distilled in narrow fractions, covering either 50° C. or 25° C. These, freed from any solid material that separated, were tested at concentrations of either 4·0 and 2·0 per cent., or 3·0 and 1·5 per cent. The results are summarized in Figs. 2 and 3.

The <250° C. fractions were the first runnings of the distillations, whilst the

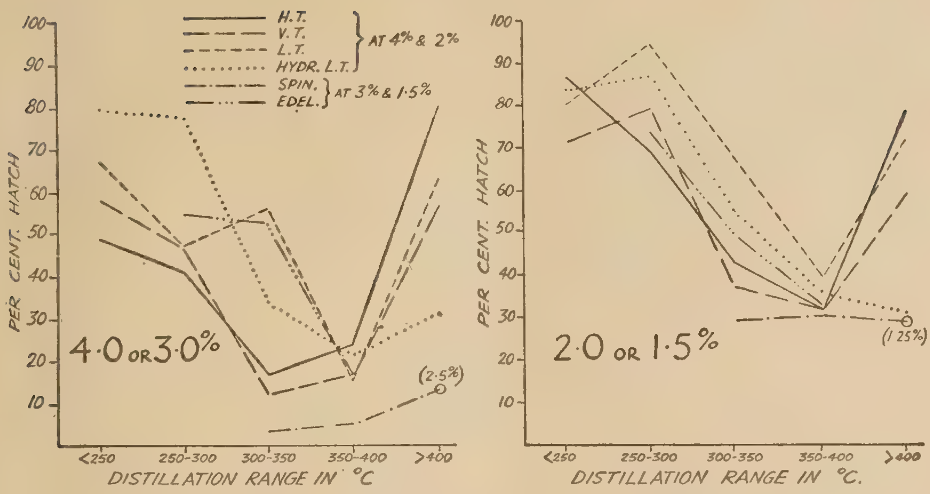

FIG. 2.
Distillation range of neutral oils plotted against per cent. hatch.

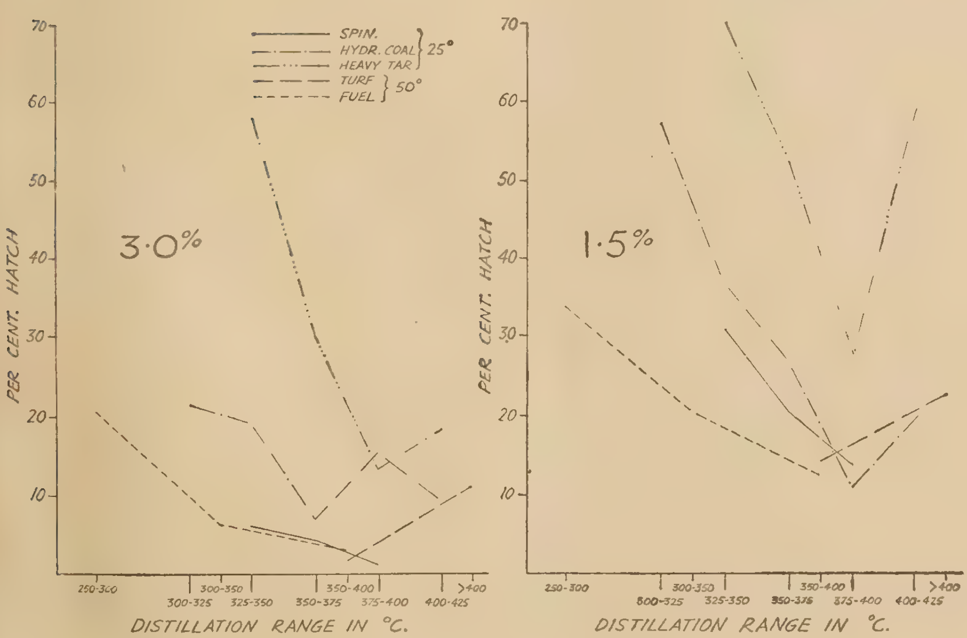

FIG. 3.
Distillation range of neutral oils plotted against per cent. hatch.

$>400°$ C. fractions were undistilled residues. Most of the latter contained pitchy matter, and several yielded poor emulsions even when dissolved in benzene, as was sometimes necessary. Neither group could be defined precisely, and the data are included in the graphs only to indicate trends. Above 400° C. toxicity fell off, this tendency being least pronounced, or even absent, in the Spin. and Hydr. L.T. oils, where there was little or no pitch. Where efforts were made to exclude pitch by distilling the $>400°$ C. fractions, the falling off in toxicity was still evident (Fig. 3). At such temperatures the effect of cracking cannot be disregarded.

All the available data on the distilled fractions between 250° C. and 425° C. are given in Table III, and the mean percentage hatches with their standard errors in Table IV.

TABLE III.

Characteristics of neutral oil fractions.

Distillation range in ° C.	Type.	Sp. gr.	Viscosity in secs. Redwood I at 70° F.	% Insoluble in Me_2SO_4
250-300	H.T.	1·049	38	0
	V.T.	0·989	40	26
	L.T.	0·877	40	30
	Hydr. L.T.	0·932	38	70
	Fuel	0·869	39	88
300-350	H.T.	1·093	85	0
	V.T.	1·020	87	30
	L.T.	1·016	91	32
	Hydr. L.T.	0·979	70	68
	Fuel	0·905	70	86
	Spin.	0·887	82	94
	Edel.	0·981	121	72
350-400	H.T.	1·133	470	0
	V.T.	1·062	534	25
	L.T.	1·069	1,157	22
	Hydr. L.T.	1·040	438	70
	Fuel	0·946	360	86
	Spin.	0·908	185	94
	Edel.	1·017	981	80
	Furf.	0·956	310	92
300-325	Hydr. Coal	1·019	74	30
325-350	Hydr. Coal	1·046	151	26
	Heavy Tar	1·093	176	2
	Spin.	0·888	89	92
350-375	Hydr. Coal	1·072	422	20
	Heavy Tar	1·118	737	2
	Spin.	0·901	149	96
375-400	Hydr. Coal	1·093	2,190	28
	Heavy Tar	1·134	2,696	2
	Spin.	0·917	316	96
400-425	Hydr. Coal	—	—	—
	Heavy Tar	—	—	—

Test No.	Date of treatment.	Distillation range in ° C.	Type.	Concentration of Oil. 4% Mean % hatch.	4% S.E.	4% Total eggs.	3% Mean % hatch.	3% S.E.	3% Total eggs.	2% Mean % hatch.	2% S.E.	2% Total eggs.	1·5% Mean % hatch.	1·5% S.E.	1·5% Total eggs.
3/37	11.ii.38	250-300	H.T.	41·1	10·8	486	—	—	—	68·8	5·1	299	—	—	—
3/37	11.ii.38		V.T.	46·2	7·5	328	—	—	—	78·9	4·0	322	—	—	—
5/37	16.ii.38		L.T.	46·7	10·8	199	—	—	—	94·4	2·1	255	—	—	—
5/37	16.ii.38		Hydr. L.T.	77·5	2·8	160	—	—	—	86·9	4·2	138	—	—	—
3/38	30.i.39		Fuel	—	—	—	20·8	7·0	224	—	—	—	33·8	6·1	226
3/37	11.ii.38	300-350	H.T.	16·8	7·5	470	—	—	—	42·5	3·8	311	—	—	—
3/37	11.ii.38		V.T.	12·5	2·7	127	—	—	—	36·5	6·5	186	—	—	—
5/37	16.ii.38		L.T.	55·5	8·4	190	—	—	—	67·5	6·6	171	—	—	—
5/37	16.ii.38		Hydr. L.T.	34·0	7·0	137	—	—	—	54·8	8·8	197	—	—	—
3/38	30.i.39		Fuel	—	—	—	6·1	1·8	307	—	—	—	20·6	5·1	279
4/37	14.ii.38		Spin.	—	—	—	3·6	2·4	288	—	—	—	28·9	5·9	273
4/37	14.ii.38		Edel.	—	—	—	52·6	14·7	192	—	—	—	49·0	8·6	146
3/37	11.ii.38	350-400	H.T.	24·0	5·3	234	—	—	—	31·3	5·5	355	—	—	—
3/37	11.ii.38		V.T.	16·4	3·3	315	—	—	—	31·4	6·2	211	—	—	—
5/37	16.ii.38		L.T.	15·2	3·5	82	—	—	—	38·9	7·1	100	—	—	—
5/37	16.ii.38		Hydr. L.T.	21·6	8·7	127	—	—	—	35·3	20·0	139	—	—	—
3/38	30.i.39		Fuel	—	—	—	2·9	1·8	405	—	—	—	12·3	1·7	303
4/37	14.ii.38		Spin.	—	—	—	5·1	3·1	528	—	—	—	30·0	2·6	201
4/37	14.ii.38		Edel.	—	—	—	16·6	9·8	193	—	—	—	31·9	9·8	202
3/38	30.i.39		Furf.	—	—	—	1·6	0·1	451	—	—	—	14·3	3·4	341
4/38	31.i.39	300-325	Hydr. Coal	—	—	—	21·3	5·7	641	—	—	—	57·2	3·7	310
4/38	31.i.39	325-350	Hydr. Coal	—	—	—	19·3	2·2	338	—	—	—	36·7	7·3	643
8/38	7.ii.39		Heavy Tar	—	—	—	57·9	4·5	173	—	—	—	70·2	7·6	346
4/38	31.i.39		Spin.	—	—	—	5·9	1·4	374	—	—	—	30·8	7·3	438
4/38	31.i.39	350-374	Hydr. Coal	—	—	—	6·9	3·2	354	—	—	—	26·8	4·7	421
8/38	7.ii.39		Heavy Tar	—	—	—	30·0	6·0	246	—	—	—	51·7	10·3	292
4/38	31.i.39		Spin.	—	—	—	4·1	2·0	400	—	—	—	20·4	6·0	302
4/38	31.i.39	375-400	Hydr. Coal	—	—	—	15·3	5·4	453	—	—	—	11·0	1·5	414
8/38	7.ii.39		Heavy Tar	—	—	—	13·3	2·1	344	—	—	—	27·7	4·5	220
4/38	31.i.39		Spin.	—	—	—	1·1	0·1	309	—	—	—	13·7	4·8	258
4/38	31.i.39	400-425	Hydr. Coal	—	—	—	9·4	4·2	558	—	—	—	20·9	4·2	346
8/38	7.ii.39		Heavy Tar	—	—	—	18·4	3·1	238	—	—	—	58·9	4·2	236

Test No.	Treatment.	Mean % hatch.	S.E.	Total eggs.
3–5/37	Control	89·5	2·0	374
3/38	..	94·9	2·8	208
4/38	..	89·2	1·8	593
8/38	..	89·7	2·0	507

In almost every case toxicity increased with increasing distillation range up to 400° C., but even when compared over a common range the oils differed widely in toxicity. Fig. 2 reveals the superiority of the Spindle oil over the tar oils, and Fig. 3 shows the Heavy Tar to be inferior to the three petroleum oils—fuel oil, spindle oil, and the furfural extract.

It is clear that viscosity had little bearing on toxicity, particularly when distillation range and specific gravity are taken into account. When selected small groups of oils were compared, specific gravity could be related to toxicity, but this relation did not hold consistently throughout the range. It was indeed sometimes positive and sometimes negative, according to the particular grouping of oils considered.

It seems that the effect of other physical and chemical characteristics on toxicity is more pronounced in the lower than in the higher distillation ranges, though even in the latter the distinction between tar and petroleum oils is evident.

An extensive statistical examination of the results in Table IV served only to confirm the obvious, namely that over the whole range of oils tested there is no consistent relation between toxicity and any physical or chemical property considered other than distillation range.

The various oil fractions tested fall into three distinguishable groups when arranged in descending order of effectiveness (Table V). The oils within a group can be regarded as of approximately equal toxicity.

TABLE V.

Miscellaneous oil fractions arranged in order of toxicity.

Group.	Oil.	Distillation ranges.
1	C.S.O.	——
	Spin.	All fractions above 300° C.
	Furf.	,, ,, 300° C.
	Fuel	,, ,, 300° C.
	Hydr. Coal	,, ,, 350° C.
2	Fuel	250-300° C.
	H.T.	All fractions above 300° C.
	V.T.	,, ,, 300° C.
	Edel.	,, ,, 300° C.
	Hydr. Coal	300-350° C.
	Hydr. L.T.	All fractions above 350° C.
	L.T.	,, ,, 350° C.
	Heavy Tar	,, ,, 350° C.
3	H.T.	250-300° C.
	V.T.	250-300° C.
	Edel.	Fraction below 300° C. (mainly 290-300° C.)
	Heavy Tar	325-350° C.
	L.T.	300-350° C.

Hydr. L.T. 300-350° C., was intermediate between groups 2 and 3; both L.T. and Hydr. L.T. <300° C., were inferior to group 3. It will be seen that no tar oil, whatever its distillation range, appears in group 1. The one glyceride oil tested

(cotton seed oil, see also Table I) compared favourably with the best of the petroleum oils. As this is a fixed oil, the relation of its toxicity to distillation range could not be examined, but it can be regarded as distilling wholly above 300° C., and in this respect comparable with the spindle oil.

TESTS OF COMPARABLE WIDER FRACTIONS.

Since distillation range appeared to be the most important single factor determining toxicity, it seemed likely that the influence of other factors would be more clearly revealed if an accurate comparison could be made of a series of oils of common distillation range. Austin, Jary and Martin (1934) had shown that a high boiling neutral tar distillate was inferior to petroleum distillates of similar distillation range in toxicity to eggs of *L. pabulinus*. For the present purpose H.T., L.T., and Spin. were selected as suitably varied types, and distilled into the longest attainable cut common to all three of them. This was delimited at the lower end by the temperature at which the spindle oil distilled freely, and at the upper by a temperature low enough to avoid cracking. These limits proved to be 320° C. and 380° C., a range that appears from Fig. 2 to cover the region of maximum toxicity. The results of tests of these fractions, given in Table VI, were examined by means of the probit transformation and the resulting dosage-mortality curves are shown in Fig. 4. Each point is based on the pooled data of five replicates, but the replication is not regarded as contributing to the degrees of freedom.

TABLE VI.

Toxicity of three oils of common distillation range 320° C.—380° C.

Test No.: 1/39. Date of treatment: 17.ii.40.

Spindle oil.			H.T. neutral oil.			L.T. neutral oil.		
Conc. % by vol.	Kill %	Total No. of eggs.	Conc. % by vol.	Kill %	Total No. of eggs.	Conc. % by vol.	Kill %	Total No. of eggs.
3·5	97·3	733	8·0	95·3	538	8·0	96·4	492
2·3	98·8	464	4·0	81·0	449	4·0	92·6	400
1·5	90·2	1,083	2·0	74·7	630	2·0	68·3	419
1·0	76·8	527	1·0	31·6	659	1·0	26·6	411
0·7	62·4	333	0·5	14·3	572	0·5	12·3	754

Since there was no evidence of departure from parallelism, the relative potencies of the oils were calculated and are shown in Table VII. This Table also contains other relevant statistical data and physical and chemical characteristics of the oils.

H.T. neutral oil did not differ from L.T. neutral oil, but spindle oil was nearly three times as toxic, a result in accord with Fig. 2. There was no evident connection of toxicity with viscosity and only a very doubtful one with specific gravity. While the amount of oil insoluble in dimethyl sulphate and the amount of unsulphonated residue seemed to bear some relation to toxicity, neither offered a sufficient explanation of the difference between the spindle oil and the others.

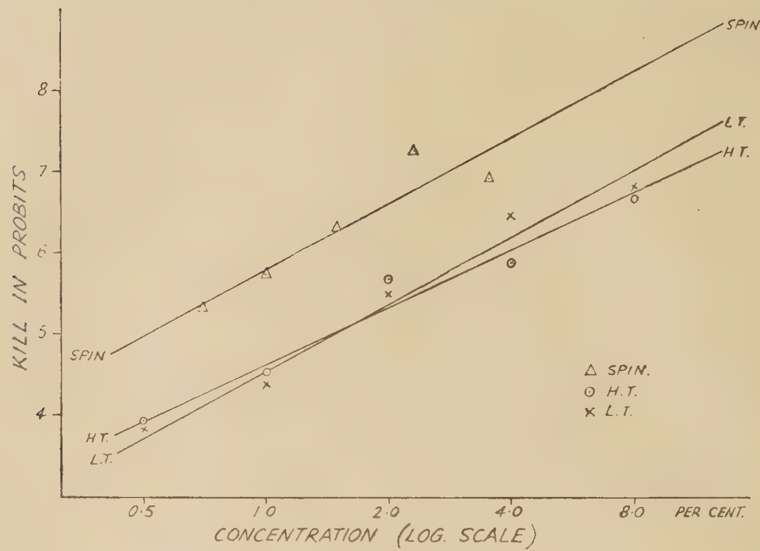

Fig. 4.

Log concentrations of neutral oils plotted against probits of mortality.

Tests of undistilled cuts.

It is clearly impracticable to use in a commercial wash very short ranges such as those tested ; the most that could be done would be to remove the less effective lighter oils. It was important, therefore, to compare the results obtained on the narrow distilled fractions with the performance of wider undistilled cuts such as might be met with in practice. These cuts would contain much of the solid material that crystallized from some of the oils on further fractionation. Moreover, the heavy,

Table VII.

Characteristics and dosage-mortality data of the three oils of Fig. 4.

	Spindle oil.	H.T. neutral oil.	L.T. neutral oil.
Viscosity	112	128	168
Specific Gravity	0·894	1·109	1·026
% insoluble in Me_2SO_4	92	0	28
% unsulph. residue	62	0	15
b	2·68 ± 0·51	2·35 ± 0·30	2·72 ± 0·24
χ^2	26·697	46·304	23·204
log. LD50	$\bar{1}$·7121 ± 0·0812	0·1463 ± 0·0463	0·1530 ± 0·0326
M	0·4371 ± 0·0575	0·0045 ± 0·0241	0
Relative Potency	2·74 ± 0·36	1·01 ± 0·06	1

pitchy matter that appears to have reduced toxicity in the > 400° C. residues would occur in a less concentrated form. In addition, the risk of cracking, with possible repercussions on toxicity, would be reduced. Accordingly the toxicity was investigated of a series of undistilled cuts of horizontal-retort neutral oil, namely > 200° C., > 250° C., > 300° C., and > 350° C. These were prepared from the neutral oil of a complete horizontal-retort distillate by removing oil up to the appropriate temperature. As each was used at two concentrations only, it was not feasible to compare their LD 50's and the results (summarized in Table VIII) were therefore submitted to analysis of variance, first transforming the percentage hatches to " Angles of Equal Information " (Bliss, 1937). In this case each of the five replicates of each treatment was used separately in the analysis.

TABLE VIII.

Toxicity of various distillation ranges of a horizontal-retort neutral tar oil.

Test No.: 6/38. Date of treatment: 3.ii.39.

Distillation range.	4% concentration.		2% concentration.	
	% hatch.	Total No. of eggs.	% hatch.	Total No. of eggs.
>200° C.	33·6	241	58·1	384
>250° C.	18·7	284	29·0	435
>300° C.	17·5	378	39·5	448
>350° C.	7·0	371	24·5	449
Controls: Hatch 91·2%. Total No. of eggs 1,359.				

Of the four cuts, that > 200° C. was significantly less toxic than any of the others even at the 0·01 level of probability; those > 250° C. and > 300° C. were indistinguishable; and the oil > 350° C. was the most toxic of all, but not quite significantly so at the usual 0·05 level.

GENERAL DISCUSSION.

This investigation has produced no evidence of a clear cut correlation of toxicity to Winter Moth eggs with any one chemical or physical property of the oils. Distillation range appears to be the most important single factor involved and, within the limits of any one type of oil, can be closely correlated with toxicity. This conclusion, arrived at by other workers in tests with straight petroleum and high temperature tar distillates, has been established for several intermediate oil types such as low temperature tar oils, hydrogenation products, and special petroleum fractions. It applies only *within* the groups of oils; and over the whole range of types when compared with each other, distillation range is quite inadequate to account for all the differences. With this qualification, the evidence supports the criteria of efficiency put forward in the official specification for dormant type petroleum oils, and no major divergence has been encountered. It is clear, however, that the specification is valid only for the normal type of petroleum oil at present in commercial

use. It does not provide a sufficient basis for discrimination between the various types of oils, and it is evident that more subtle factors not yet amenable to specification are involved in the ovicidal action of these oils.

In general, the tar oils are much less toxic than petroleum oils of similar distillation range, while the hydrogenated oils are intermediate. Any attempt to explain the toxicity of these oils in terms of individual chemical compounds is frustrated by the extreme paucity of information available about such individuals in all but the horizontal retort high-temperature distillate. The most that can be attempted, therefore, is to relate toxicity to oil types.

The results provide detailed evidence in support of the tentative suggestion of Austin, Jary and Martin (1934) that the chemical nature of the oil is important in determing toxicity ; the most highly aliphatic oils, i.e. those least soluble in dimethyl sulphate, were more toxic than the most highly aromatic. This relation is less evident, however, among the oils of intermediate type where, for example, Hydr. Coal approached the petroleum oils in effectiveness, but the more highly aliphatic Hydr. L.T. was consistently less toxic. The L.T. oil (30 per cent. insoluble in dimethyl sulphate) was less toxic, especially in the lower distillation ranges, than the wholly aromatic H.T. oil. On the other hand, in the one test where comparison is possible, the furfural extract was much more toxic than the more highly aromatic Edeleanu extract. It may be that further examination of the composition of the aliphatic portions of the oils would explain some of these anomalies. It must be remembered that the term aliphatic covers all the non-aromatic components and thus includes hydroaromatic (naphthenic), paraffinic, and unsaturated types. Thus the tar oils are predominantly aromatic and the spindle oil mainly paraffinic. The hydrogenation products are intermediate in type, but Hydr. L.T. contains a greater proportion of hydroaromatic constituents than does Hydr. Coal. This evidence indirectly suggests that the most toxic constituents of the oils may be located in the paraffinic fraction. This would explain the relatively small increase in toxicity resulting from hydrogenation of low temperature tar ; for, while this process greatly increases the content of aliphatic constituents, this increase is due mainly to the formation of hydroaromatic compounds.

On the basis of the summarized classification given in Part I (page 4) there is a suggestion from the biological results that aromatic, hydroaromatic (naphthenic), and paraffinic oils may stand in that order of increasing toxicity to Winter Moth eggs. If this hypothesis is correct it may be regarded as an amplification and extension of the conclusion arrived at by Pearce, Chapman and Avens (1942) in relation to Fruit Tree Leaf-roller, that within a series of petroleum oils the more paraffinic are the more toxic. It is clear that Tutin's view that toxicity is independent of the nature and source of a tar oil cannot be extended to apply throughout the range of oils considered here, but much more detailed information about the oils will be necessary before the hypothesis postulated above can be substantiated or disproved.

ACKNOWLEDGMENTS.

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of the merits of various methods of presenting these results. They gratefully acknowledge gifts of materials from the various organizations named in Part I of this series. Many individuals in these and other organizations have furnished information and opinions without which it would have been impossible to prepare this paper.

SUMMARY.

A selection of tar and petroleum oils covering a very wide range of chemical types was tested against eggs of the Winter Moth (*Operophtera brumata* L.). The first broad survey showed that of several physical and chemical characteristics determined for each oil, distillation range, especially the content of oil distilling above 300° C., was most closely related to toxicity, but did not wholly explain it. Tests of distilled fractions of the neutral oils showed that for any one oil, toxicity increased with distillation range up to about 400° C., but varied widely between similar distillation ranges of different oils. The petroleum oils in general were much more toxic than the tar oils. Over the range of oils as a whole, though with some exceptions, it appeared that the proportion of aliphatic material in the oil, as judged by the percentage insoluble in dimethyl sulphate, might be a considerable factor in determining toxicity. Indirect evidence suggested that paraffinic components were probably more toxic than naphthenic. Viscosity seemed to have little connection with toxicity, and specific gravity appeared to be connected only within small, closely related groups of oils. The phenols and bases isolated from the tar oils were not sufficiently toxic to contribute materially to the toxicity of the whole oils nor, on the other hand, were they present in sufficient quantity to reduce the toxicity by dilution.

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THE GUAIACOL-HYDROGEN PEROXIDE AND BENZIDINE-HYDROGEN PEROXIDE COLOUR REACTIONS OF THE BEAN *(PHASEOLUS VULGARIS* L.) POD

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Introduction.

In the preparation of dehydrated vegetables the inactivation of enzymes is necessary, otherwise discoloration and loss of flavour result; or, even worse, unpleasant "off" flavours and odours develop. The enzymes are inactivated by subjecting the vegetables for the necessary length of time to steam, to hot or boiling water, or to certain hot or boiling solutions—a process known as blanching. Little is known of what enzymes are responsible for the various changes involved in deterioration, and it has been necessary to use the destruction of one particular enzyme as an indicator of adequate blanching. The one chosen must be widely or universally present in plant tissues and it must not be destroyed too readily by heat. Peroxidase fulfils these requirements and has been increasingly used for blanching tests.

Under factory conditions, a rapid and simple method of testing is necessary, and use has been made of the development of a specific colour when a solution of a peroxidase indicator (enzyme substrate) together with a solution of hydrogen peroxide are added to the material to be tested. The indicators now most commonly used are benzidine and guaiacol. Because of its widespread use, the reliability of this method is important, and results obtained with vegetables, especially beans, have raised some doubts on this point especially in regard to benzidine.

One of the difficulties connected with this method is that the term peroxidase is a generic one. The existence of peroxidases has been demonstrated in horseradish (Theorell, 1940), and probably more than one occurs both in cabbage and turnip (Schwimmer, 1944); but the presence of more than one in a plant organ has been postulated in some instances solely on the basis of colour reactions with hydrogen peroxide and peroxidase indicators. Thus, Keeble and Armstrong (1912) reported that in sections of leaves, petals and stems of *Primula sinensis* the vascular tissues alone developed a lavender blue colour with α-naphthol and hydrogen peroxide. Benzidine and hydrogen peroxide, however, induced " . . . a rich brown uniform coloration of the surface layer of the petals, which coloration extends also to the veins" (p. 216). Neither indicator affected tissues other than the epidermis and vascular bundles. Bedford and Joslyn (1939), when investigating the blanching of beans, noted that a blue colour was given by benzidine and hydrogen peroxide when guaiacum and hydrogen peroxide had ceased to give a colour reaction.* Unfortunately, these workers give no information as to the particular tissues involved. Both these contributions assume the existence of more than one peroxidase on

* No attempt has been made at an extensive presentation of different blanching times for the same vegetable with different indicators. The paper by Bedford and Joslyn here cited is to be regarded as illustrative and has been chosen since it deals with beans.

the evidence of different qualitative colour reactions. Keeble and Armstrong deduce the presence in *Primula sinensis* of an "epidermal" and of a "bundle" peroxidase; Bedford and Joslyn refer to a "benzidine peroxidase" and a "guaiacum peroxidase".

Phaff and Joslyn (1943) recorded their own observations and quoted those of Mergentime on blanching tests with peas, beans (Joslyn), maize (Mergentime, Phaff and Joslyn) and potatoes (Phaff and Joslyn). In some of these tests, the blue colour developed with guaiacum and hydrogen peroxide was used and in others use was made of the reddish-brown colour obtained with hydrogen peroxide and guaiacol. These observations indicated that the "apparent rate of thermal inactivation of the vegetable peroxidases" differed in different parts of the same vegetable. The rate of thermal destruction of a colour reaction was much greater in the cotyledons of peas and beans than in their skins, and in the inner parts of the maize grain than in outer parts, especially at the base in the region of attachment to the cob. A reddish brown colour with guaiacol and hydrogen peroxide was also recorded (p. 51) "around the eyes and vascular bundles" of potatoes when this colour reaction had been destroyed by heat in other parts. This usually occurred in potatoes that had been "physiologically damaged". Phaff and Joslyn referred to these persistent colour reactions more cautiously as due to "thermostable catalysts".

The results obtained with beans in this laboratory were essentially of the type obtained by Bedford and Joslyn except that, as will be seen later, the benzidine-hydrogen peroxide reaction was found to be much more thermostable than was established by these workers, who noted its persistence after five minutes at 100° C., when the guaiacum reaction had already been destroyed.

Scope of investigation.

It was found that after the capacity of bean tissues to give a brown colour with hydrogen peroxide and guaiacol had been lost, a blue colour was still developed in certain tissues on adding hydrogen peroxide and benzidine. This blue colour was identical with that developed on adding the same reagents to fresh bean tissues. A brown colour was also obtained with guaiacol and hydrogen peroxide in certain tissues when the capacity to give a general browning of all tissues had been destroyed. It was decided to pursue the matter further and an investigation was accordingly carried out to determine what tissues gave the relatively thermostable guaiacol-hydrogen peroxide reaction and, in particular, to ascertain exactly where the thermostable benzidine-hydrogen peroxide reaction occurred. It was also clearly necessary to ascertain whether the colour reactions obtained might not be due to extraneous factors; hence tests were made to ascertain the effect of the purity of the water used for blanching and of the presence of traces of acetanilide in the hydrogen peroxide used.

Sources of materials.

The sources of the bean samples used were:

(1) Cape Town retail vegetable market and shops.
(2) Private garden.
(3) Varkensvlei Experimental Vegetable Farm, Cape Flats.
(4) Turf Hall Farm, Philippi, on the Cape Flats.

Beans from the first source were of unknown varieties, but most of those from the other three were of the following named varieties: Canadian Wonder, Kentucky Wonder, Burpee Stringless, Long Tom, Tender Green, Chestnut Greenpod, Ferry's Plentiful, Extra Early Red Valentine and Kudu Stringless.

QUALITATIVE PEROXIDASE TESTS.

One per cent. alcoholic solutions of benzidine (B.D.H. and May and Baker) and guaiacol (B.D.H.) were used. Samples of recrystallized benzidine were also tested. For a few tests a ten per cent. solution of gum guaiacum was used.

The material was placed in a porcelain evaporating dish and more or less covered with about equal amounts of peroxidase indicator and hydrogen peroxide solutions. The presence or absence of an instantaneous colour reaction was noted and a record of colour development was made after $3\frac{1}{2}$, 5 and 15 minutes. These time intervals were based on various routine practices used in testing blanching efficiency. If no colour developed within five minutes, usually none was noted after 15 minutes. In some cases colour developed after a much longer interval. With fresh material the colour developed instantaneously or rapidly with guaiacol and benzidine.

The concentration of hydrogen peroxide used by different investigators of vegetable processing in testing for peroxidase has varied, but it has been found by biochemical research that with too high a concentration of hydrogen peroxide, peroxidase does not give a colour with peroxidase indicators. This is because compounds are then formed between hydrogen peroxide and the peroxidase complex which are not catalytically active. An active compound is formed with lower concentrations of hydrogen peroxide (Theorell, 1942).

The writers used concentrations of hydrogen peroxide ranging from 3·0 to 0·1 per cent., 0·1 to 0·3 per cent. being found most satisfactory, especially with guaiacol. Although it was often observed with this indicator that a brown colour developed with 0·1 or 0·15 per cent. solutions of hydrogen peroxide when no colour was obtained with solutions of ten times these concentrations, in other cases colour reactions were obtained with high concentrations including 40 per cent., which was the highest concentration tested. It is beyond the scope of this paper to analyse this matter in detail, but it may be mentioned that the results obtained are affected by the length of blanching and that the character of the colour reactions obtained with higher concentrations of hydrogen peroxide was unlike that obtained with solutions of the order of 0·1 to 0·3 per cent. With higher concentrations there was a greater development of brown colour on the outer surface of the pod, this colour tending to be darker and less reddish than that typically developed with weak solutions of hydrogen peroxide; brown colour was developed in differentiated tissues, but was absent or very poorly developed in the endocarp (Fig. 1, EN); and as contrasted with low concentrations, the testing fluid did not become brown. The blue colour developed with benzidine was, in general, less affected by the concentration of hydrogen peroxide, although the amount of colour was less both with very high and very low concentrations. A 1·0 or 1·5 per cent. solution in some cases gave decidedly more colour than a 0·15 per cent. solution. As with guaiacol, high concentrations tended to suppress colour development in the endocarp. The occurrence of colour reactions over such a wide range of hydrogen peroxide concentration is interesting in view of the restriction of a colour reaction to a low concentration of hydrogen peroxide with peroxidase extracts.

Blanching.

The beans were cut across with a stainless steel knife (or a cutting machine of stainless steel) into slices about one half to one centimetre thick. The slices were then washed in water, enclosed in boiled muslin and put into a beaker of boiling water. For some of the earlier tests, small, fine meshed phosphor-bronze or tin-plated wire baskets were used instead of muslin, but these proved unsatisfactory.

The proportion of beans to water was 100 gm. to 250 c.c. During prolonged blanching additional boiling water was added periodically to maintain approximately the original bulk of fluid. On immersion in boiling water the temperature fell and the water ceased to boil, so the blanching period was reckoned from the time of resumption of boiling, which usually occurred within two minutes.

Tests were made with both tap and distilled water, and the latter included a sample that had been three times redistilled in an apparatus made entirely of glass.

Microscopical Preparations.

Blanched material is too soft for making hand sections and thus sections of fresh pods were cut into 70 per cent. alcohol (to eliminate air bubbles), removed, washed twice with distilled water and blanched for the required time. After blanching, the sections were generally mounted in water, the peroxidase indicator was drawn in under the cover glass and colour development observed, the colour pattern being again examined after a few minutes. Sometimes the sections were first put into one or both testing fluids and then mounted. The blue colour produced by benzidine changed to brown after a time, and brownish colours developed in certain tissues during blanching in water. Untreated blanched sections were examined as controls along with the treated ones. Observations were made under the microscope both with transmitted light and with dark background illumination.

Comparative tests showed that immersion in 70 per cent. alcohol followed by washing in distilled water did not affect the distribution of colour developed on adding the peroxidase indicator and hydrogen peroxide, although sometimes its intensity was slightly increased.

Effect of Acetanilide Stabilizer.

Commercial hydrogen peroxide contains small amounts of a stabilizing substance ; that used contained acetanilide, in very small amount—of the order of 1 in 10,000 in a 3 per cent. solution. This would give concentrations of 1 in 300,000 and 1 in 200,000 in 0·1 and 0·15 per cent. solutions, respectively. Tests were also made with solutions prepared from 3 per cent. hydrogen peroxide containing 0·1 per cent. acetanilide, which is about ten times the amount generally used.

Parallel tests were carried out with stabilized and unstabilized hydrogen peroxide. Supplies of the latter were prepared at the Government Chemical Laboratories, Cape Town, at Heynes Mathew's Laboratory, Cape Town, and by the writers themselves. Colour development in blanched and partially blanched beans was tested with both guaiacol and benzidine using hydrogen peroxide solutions of different concentrations ; blanching times of an hour and half an hour were included. The results of numerous tests showed that, for practical purposes, the quality and intensity of the colour developed with both reagents were not essentially affected by the presence

of acetanilide in hydrogen peroxide. This is illustrated in Table I, which shows that in three cases slightly less, and in a fourth case slightly more, colour was developed in differentiated tissues in the presence of acetanilide. It should be stressed, however, that where a difference occurred it was very slight and could be distinguished only on careful scrutiny. Thus, in all cases, the colour response was of the same order whether acetanilide was present or not. Experiments 5 to 12 are especially significant since the concentration of acetanilide in a 3 per cent. stock solution was 0·1 per cent. instead of 0·01 per cent.

It has been claimed by Wiegand, Madsen and Price (1943) that the presence of acetanilide renders hydrogen peroxide unsuitable for peroxidase tests, although they do not present evidence in support of their statement. It should be emphasized however, that although the presence of acetanilide was without effect (or appreciable effect) on the more thermostable colour reactions of bean tissues, it is not maintained that its presence is unimportant with vegetables other than beans, with peroxidase indicators other than guaiacol and benzidine, or in testing tissue extracts and solutions of peroxidase extracts. Tests with clear aqueous extracts of partially blanched beans showed that, in fact, acetanilide at the higher concentration tested (0·1 per cent. in 3 per cent. stock solution) may under certain circumstances appreciably lessen the intensity of colour developed, especially when guaiacol is used.

TABLE I.

Effect of acetanilide stabilizer in dilute hydrogen peroxide solutions (0·1 to 0·3 per cent.) on development of relatively thermostable colour with guaiacol, and thermostable colour with benzidine.

Expt. No.	Blanching time at 100° C. (minutes).	Indicator used.	Colour development.	
			Stabilizer absent.	Stabilizer present.
1.*	6	Guaiacol	xxx	xxx
2.	3	,,	xxxxx	xxxx
3.	6	,,	xxxxx	xxxxx
4.	40	Benzidine	xxxxx	xxxxx
5.	10	Guaiacol	xxxxx	xxxxx
6.	30	Benzidine	xxxx	xxxxx
7.	30	,,	xxxxx	xxxxx
8.	5	Guaiacol	xxxxx	xxxx
9.	10	,,	xxxx	xxxx
10.	10	,,	xxxxx	xxxxx
11.	15	,,	xxx	xxx
12.	15	,,	xxxx	xxx
13.	8	,,	xxxxx	xxxxx

KEY.—x=Very slight (hardly discernible) colour; xxx=slight coloration; xxxxx=normal colour pattern.

* Long Tom beans from Philippi were used for Expt. 1., otherwise, different lots of market and shop beans of unknown varieties were used.

ANATOMY OF THE BEAN POD.

In describing the anatomy of the bean pod, Stark and Mahoney's (1942) terminology has been adopted. If a transverse section of an unripe pod cut a little

above or below a seed is examined, the pod wall is seen to consist mainly of an outer green region and an inner colourless one. The latter is the endocarp (Fig. 1, E.N.), but the green region is more complex, consisting of the exocarp (EX) and the outer mesocarp (om). The " strings " are tissues of the dorsal and ventral sutures (DS and VS). The boundary between the chlorophyllous region and the endocarp is marked by a narrow zone of denser tissue, the " fibrous layer " or inner mesocarp (im), which is succeeded on the outside by the outer mesocarp (om) in which lie a number of

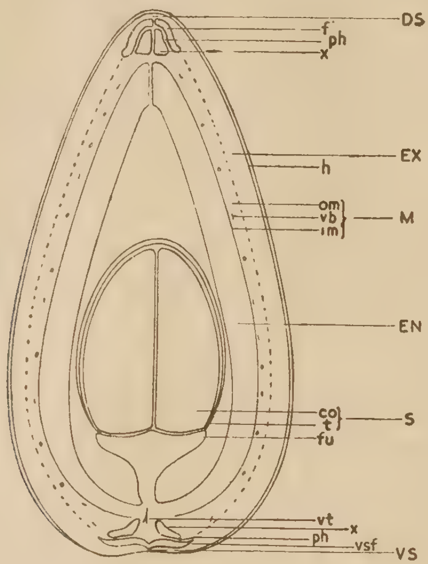

FIG. 1.

Diagram of transverse section of the bean (*Phaseolus vulgaris* L.) pod.
co, cotyledon ; DS, dorsal suture ; EN, endocarp ; EX, exocarp ; f, fibres ; fu, funicle ; h, hypodermis ; im, inner mesocarp (fibrous layer) ; M, mesocarp ; om, outer mesocarp ; ph, phloem ; S, seed ; t, testa ; vb, vascular bundle ; VS, ventral suture ; vsf, ventral suture fibres ; vt, vascular trace ; x, xylem.

vascular bundles (vb). The outer mesocarp passes on the outside into the exocarp (EX). The exocarp and endocarp are bounded respectively by the outer and inner epidermis.

In the unripe pod the endocarp is of relatively large bulk especially in the intervals between the seeds, but as the pod matures the endocarp becomes thinner by the loss of water and collapse of its cells. Large thin-walled cells and air spaces constitute this tissue which contains very little starch in contrast to the outer mesocarp and exocarp.

The vascular supply to the ovary, as stated by Doutt (1932), consists of a ring of twelve bundles. The dorsal and ventral sutures are each partly formed by two of these, the dual nature being clearly discernible in transverse section in the ventral (broader) suture. The other eight bundles were shown by Doutt to branch and anastomose in the mesocarp and to connect up with the sutures. Each string is comprised of two vascular bundles, on the outside of which is a group of pericycle

fibres, which become progressively lignified with age and sometimes brownish in colour. When a bean is stringed it is these fibres, or most of them, together with possibly part of the phloem, that are removed. Beyond the fibres and at their sides are patches of strongly thickened collenchyma. The vascular bundles are of typical dicotyledonous type with proto- and metaxylem vessels and wood parenchyma; outside is a layer of phloem. The ventral suture supplies the seeds, the branches coming alternately from each of the two bundles. The branches given off by the suture bundles traverse the side walls and, as seen in section, are small, containing annular and spiral vessels and phloem. They form an interrupted ring in the outer mesocarp.

The exocarp is composed of chlorophyll-containing cells bounded on the outside by an epidermis with stomata, and bearing—in the sections of Long Tom and the unnamed market beans examined—mostly two-celled hooked hairs. Below the epidermis there is a layer of colourless, elongated cells, the hypodermis (Fig. 1, h).

The inner mesocarp (or fibrous layer) is the parchment layer of Kooiman (1931). Since the development of stringless beans (in which the pericycle fibres do not become lignified), the nature of this layer has become the principal criterion by which the suitability of bean varieties for canning is judged. It consists of several rows of fairly small, closely packed fibres, with thickened walls, which do not run parallel to the length of the pod but diagonally from the dorsal suture towards the apex of the pod.

Attempts were made to determine the nature of the cell walls of the various tissues. The walls of the vessels and of the pericycle fibres of the sutures were lignified. Suberin was found only in the cuticle and the base of the epidermal hairs. The walls of the remaining tissues, with the exception of the fibrous layer (inner mesocarp) were of cellulose. In young pods the walls of the inner mesocarp cells consist of cellulose (Schultz's solution) and in some lots of old pods this fibrous layer was lignified. Lignin was indicated by the development of a red colour with phloroglucinol and hydrochloric acid in the cold. Lignification of the inner mesocarp has also been reported by Currence (1930). Stark and Mahoney, on the other hand, reported that in Giant Stringless Green Pod and Bountiful beans, thickening of the walls of the inner mesocarp was due to hemicellulose. Repeated but unsuccessful attempts were made to find hemicellulose in the fibrous layer of the bean samples studied, using the phloroglucin-hydrochloric acid test for xylan-araban hemicelluloses. It may be mentioned that Stark and Mahoney comment that the amount of the thickening is affected by external conditions, high rainfall and low temperatures being unfavourable to the process. Currence's results suggest that not only the amount, but possibly the nature of the thickening may be affected by external conditions. Varietal differences must also be borne in mind.

COLOUR REACTIONS OF FRESH TISSUE.

Fresh bean pod and seed tissues (Kentucky Wonder and shop beans) were found to give no colour reactions in the absence of hydrogen peroxide when immersed in 1 per cent. solutions of guaiacol or benzidine. In this connection attention may be directed to the statement by Bedford and Joslyn (1939, p. 756) that: "Apparently the cut string beans contain an enzyme system capable of bluing guaiacum directly." Tests were made with guaiacum by the present writers. In some cases no colour

reaction was obtained, but in other cases a fresh but unrefluxed 10 per cent. solution gave a not very marked development of blue colour in "string", inner mesocarp and inner epidermis in five minutes. When the test was repeated with pods from the same consignment, but using a 10 per cent. guaiacum solution that had been refluxed for about half an hour with Norit charcoal, there was at best only a very slight development of blue colour in "string" and inner mesocarp tissues after an interval of 15 minutes; a colour reaction was, indeed, practically absent.

In the presence of hydrogen peroxide all the pod tissues gave a brown or reddish brown colour with guaiacol, a greenish blue colour with guaiacum and a blue colour with benzidine. The outer surface of pod segments showed little or no colour, except at the sutures, unless a high concentration of hydrogen peroxide was used. With dilute solutions of hydrogen peroxide, however, the epidermal cells coloured up on transverse cut surfaces in direct contact with the reagents. As for the seed, except for the hilum, the outer surface of the testa showed little or no colour reaction, while its inner surface showed a marked one. The cotyledons, radicle and plumule showed greater or less colour except for very rudimentary plumules, which showed none. These reactions were obtained not only with bean segments and dissected seeds, but also with isolated samples of the various tissues. Colour development was rapid and often instantaneous.

Microscopical examination (rendered somewhat difficult by the effervescence resulting from the interaction of catalase and hydrogen peroxide) showed a general colour reaction of the cell sap as well as of the walls of those tissues (to be dealt with later) that showed the thermostable benzidine-hydrogen peroxide reaction.

Tests with Blanched Tissues.

Certain bean pod tissues invariably developed a blue colour with benzidine and hydrogen peroxide after blanching periods of up to an hour; longer periods were not tested; after much shorter periods no brown colour was developed with guaiacol and hydrogen peroxide.

The results of tests with tap water, distilled water and triple distilled water were identical, irrespective of the kind of water used.

Table II summarizes the results of observations relating to the effect of blanching time on the destruction of the relatively thermostable guaiacol-hydrogen peroxide reaction of bean pods. They were obtained chiefly when investigating relative blanching times with different solutions. For most of the bean samples two-minute blanching intervals were used, water blanched material being used as a control. One set of tests only was carried out with consignments 7 and 8 and there was a complete absence of brown colour on adding hydrogen peroxide and guaiacol. The symbol $<$ indicates that the shortest period necessary to attain this result was not determined; $>$ indicates that the longest blanching period tested did not completely eliminate the brown guaiacol-hydrogen peroxide reaction; the use of brackets indicates all but complete destruction, since a brown colour developed on only a small proportion of tissues in a small proportion of pod slices. In regard to the use of guaiacol and hydrogen peroxide, one feature deserves attention. Injured tissues may show colour when otherwise no colour would develop. Even very small hardly noticeable injuries may cause colouring in this way, but the rate of colour development is usually slow.

With both benzidine and guaiacol a general colour reaction of all tissues was rapidly destroyed on boiling. It was difficult to determine precisely what length of time of blanching sufficed to destroy this general colour completely. Usually it was destroyed more or less completely within three to five minutes, although the greater

TABLE II.

Results of blanching experiments relating to the destruction of the relatively thermostable guaiacol-hydrogen peroxide reaction of various bean samples.

Origin and variety. Consignment of beans :	Blanching time (minutes) for destruction of relatively thermostable colour with guaiacol and H_2O_2.
Market and shops (unnamed varieties).	
Consignments 1 and 2	>10
Consignment 3 (three duplicate samples)	(15)
Consignments 4 and 6	> 8
Consignment 5	> 6
Consignment 7	<12 (4-min. lag)*
Consignment 8	< 8 (3-min. lag)
Private garden.	
Long Tom 9	(6)
Unnamed 10	15-20
Varkensvlei experimental farm.	
Extra Early Red Valentine 11	8-10
Ditto, after 4 additional days at 40° F.	6-8
Ferry's Plentiful 12	8
Ditto, after 7 additional days at 40° F.	6
Chestnut Greenpod 13	(8)
Ditto, after 9 additional days at 40° F.	(6)
Kudu Stringless 14	>6
Tender Green 15	6
Long Tom 16	10-15
Burpee Stringless 17	(15)

* Where lag period is not given it did not exceed 2 minutes.

part of it was destroyed more quickly. A general colour persisted longer in the stalk and apical regions of the pod. Two phases in loss of ability of bean pods to give a brown colour reaction with guaiacol can thus be recognized. Firstly, the disappearance, broadly speaking, of a brown colour in parenchymatous tissues and secondly in fibrous and vascular tissues. After a few minutes boiling, the outer surfaces of the pod may show brown patches, especially near the sutures, provided blanching is not continued long enough to destroy the guaiacol-hydrogen peroxide reaction completely. In some cases at least, this surface colour was the last to disappear, persisting for a short time after the destruction of colour in other regions. In this paper, however, chief stress is laid on the complete loss of colour with guaiacol as compared with its persistence with benzidine.

If a solution of benzidine only is added to bean pods which have been boiled for half an hour, a blue colour is obtained only after the tissues have remained immersed in the solution for half an hour to an hour. Thin, slight zones of bluish

colour are then seen in the strings, the inner mesocarp (fibrous layer) and the vascular bundles of the outer mesocarp. These are also the tissues that become coloured on adding both hydrogen peroxide and benzidine to pod slices blanched for half an hour or an hour. When hydrogen peroxide is added, however, a blue colour develops within a few minutes; it is more intense and the zones of colour are broader. The benzidine-hydrogen peroxide reaction of the hypodermis is more heat stable than that of the parenchyma, but is not given by bean slices boiled for half an hour.

Sections were examined under the microscope to determine more precisely the

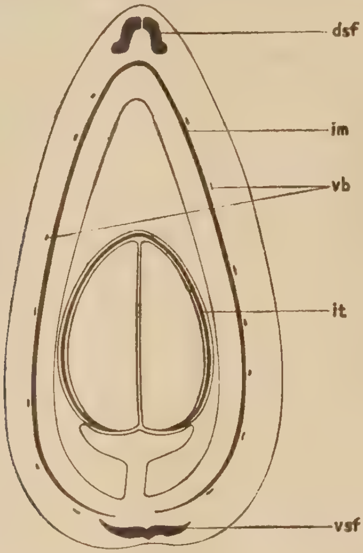

FIG. 2.

Diagrammatic cross section of bean pod showing, in heavy black, tissues giving a thermostable benzidine-hydrogen peroxide reaction.
dsf, dorsal suture fibres; im, inner mesocarp (fibrous layer); it, inner surface of testa; vb vascular bundles of outer mesocarp; vsf, ventral suture fibres.

location of the blue colour developed on adding hydrogen peroxide and benzidine to bean pods subjected to long blanching periods (Fig. 2), and it was found in the following tissues:

(1) The fibres of the dorsal (dsf) and ventral (vsf) suture bundles, the thick walls of these fibres being prominently coloured.

(2) The inner mesocarp (fibrous layer) (im), which developed a blue colour irrespective of the stage of development and whether the walls were of cellulose or were lignified.

(3) The vascular bundles (vb) of the outer mesocarp, in which the blue colour seemed to be located around the xylem and to some extent, at least, in the walls of the vessels.

It should be stated that not all the vascular bundles of the outer mesocarp necessarily showed the blue colour. No colour was observed in the vessels of the suture bundles.

The inner surface of the testa (it) may also give a thermostable blue colour, but it is more difficult to observe since the testa often becomes greyish blue on ripening and especially after prolonged boiling. A thermostable blue colour with benzidine and hydrogen peroxide, however, is not invariably obtained.

Both young and old pods were examined and gave similar results. While young pods gave a more marked colour reaction after short blanching periods, the older ones gave a deeper blue colour in all the tissues indicated above, after prolonged boiling.

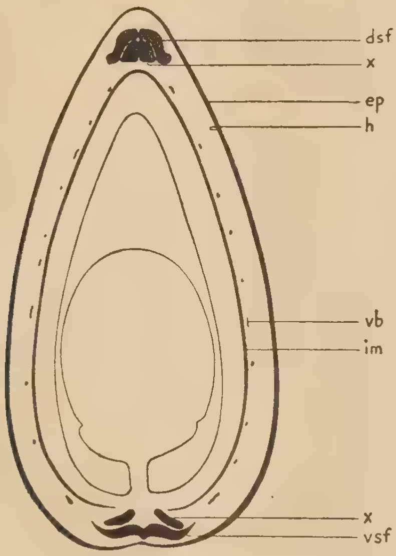

Fig. 3.

Diagrammatic cross section of bean pod showing, in heavy black, tissues giving a relatively thermostable guaiacol-hydrogen peroxide reaction.
dsf, dorsal suture fibres ; ep, epidermis ; h, hypodermis ; im, inner mesocarp (fibrous layer) ; vb, vascular bundle of outer mesocarp ; vsf, ventral suture fibres ; x, xylem of suture bundles.

Bean pod slices blanched for half an hour give no colour reaction when immersed in guaiacol solution for half an hour or an hour.

Sections were examined to determine in which tissues the guaiacol-hydrogen peroxide reaction was most stable. It will be seen from Fig. 3 that those tissues showing a thermostable benzidine-hydrogen peroxide reaction also showed a relatively stable guaiacol-hydrogen peroxide reaction. The latter reaction was also shown by :

(1) The xylem vessels (x) of the suture vascular bundles (dsf and vsf).
(2) The hypodermis (h).
(3) The epidermal cells (ep).

In the first two tissues the colour was a feature of the walls only, but the contents of the epidermal cells became coloured, although there seemed to be some browning of the cuticle. In the small bundles of the outer mesocarp (vb) the colour was most apparent in the xylem. The hypodermis (h) showed least colour.

DISCUSSION.

Considered in relation to blanching time, the colour reactions of bean pods with guaiacol and benzidine can be classified into three groups :

(1) A thermolabile colouring of parenchymatous tissues with both indicators, in the presence of hydrogen peroxide.

(2) A relatively thermostable brown colour reaction of certain tissues with guaiacol and hydrogen peroxide.

(3) A thermostable benzidine-hydrogen peroxide blue colour reaction of certain differentiated tissues.

The thermolabile reaction affects the cell contents, whilst, with the possible exception of the guaiacol-hydrogen peroxide reaction of the epidermal cells, the relatively thermostable guaiacol-hydrogen peroxide and the thermostable benzidine-hydrogen peroxide reactions affect the walls of differentiated tissues. If there should be thermolabile colour reactions of the walls of differentiated tissues, these would be masked by the more thermostable reactions. The thermolabile reactions can be accepted as true peroxidase effects. The nature of the relatively thermostable guaiacol-hydrogen peroxide reaction needs further elucidation ; here the thermostable benzidine-hydrogen peroxide colour is the chief point of interest. Although relatively thermostable peroxidases have been noted by previous workers much shorter blanching periods than one hour appear to have been used.

An alternative explanation is that this thermostable blue colour is caused by the presence in certain bean tissues of some substance or substances of non-enzymic nature. On the other hand, markedly thermostable enzymes have been reported. A paraphenolase isolated from pear leaves has been shown by Samisch to withstand a temperature of 100° C. for 30 minutes. He comments : " . . . the heat stability . . . would lead one to believe that it is an inorganic catalyst ". (Samisch, 1937) ; he also gives references to reported thermostable oxidases in other plants.

The data presented in this paper indicate that the presence of a thermostable benzidine-peroxidase is not the reason for the thermostable benzidine-hydrogen peroxide reaction of the bean. Peroxidase is a cell sap enzyme (Mangenot, 1928), but the blue colour under discussion is developed not only in tissue walls (largely lignified tissue) but also in the walls of such dead tissues as the pericycle fibres of the dorsal and ventral sutures, the vessels of the vascular bundles of the outer mesocarp and the lignified fibres of the fibrous layer of fully mature pods. It should be borne in mind, however, that the walls of the inner mesocarp fibres develop a thermostable blue colour with benzidine and hydrogen peroxide irrespective of the presence or absence of living contents. Furthermore, the development of colour when a benzidine solution is added to the pod tissues, takes place over a wide range of hydrogen peroxide concentrations.

The guaiacol-hydrogen peroxide reaction is less clear. With the exception of the epidermis this reaction affects only the walls of differentiated tissues, but it is affected to a greater extent by the concentration of the hydrogen peroxide.

The question arises as to the application of these observations to the use of

guaiacol and benzidine as indicators of peroxidase in bean tissues. Two conclusions have been arrived at. Firstly, benzidine is not suitable, since the benzidine-hydrogen peroxide reaction in the differentiated tissues is very thermostable. Similar results were obtained with cabbage, but with it the reaction was less thermostable. When the tests reported in this paper had already been completed, a paper on the blanching of beet by Madsen, Litwiller and Wiegand (1944) came to notice. These workers found that blanching of sliced beet at 240° F. for 10 minutes was insufficient to destroy the benzidine-hydrogen peroxide reaction, although 15 minutes was sufficient. They consequently questioned the suitability of benzidine as a peroxidase indicator for beet and suggested (p. 86) " . . . that there is present in the vegetable a thermostable substance (or substances) that can catalyze the H_2O_2-benzidine reaction to give the colour test ".

Secondly, guaiacol also, when added with hydrogen peroxide to bean pod tissues, is unsuitable as an indicator of blanching adequacy. It is true that for some lots, even assuming that the total destruction of the guaiacol-hydrogen peroxide colour reactions implies more than peroxidase destruction, the blanching time interval between destruction of the general colour-reaction and its total destruction is not so long as to make this indicator useless. For other bean lots, however, this interval is far too long. General experience, as well as tests carried out in this laboratory, indicate that blanching periods of 15 and 20 minutes are quite unnecessary for the preparation of dehydrated and canned beans which will retain their qualities during long storage.

The question suggests itself as to whether the destruction of the general colour reaction with both guaiacol and benzidine may not be used as a criterion of peroxidase destruction. This would seem to be sound in principle, but it would be very difficult in practice, since the regions of relatively thermostable colour with guaiacol and the thermostable colour with benzidine make up a fair proportion of the tissues. Thus, careful scrutiny becomes necessary, as the intensity of general coloration is quickly reduced on blanching. The soundness of the method may also be questioned on the basis of possible outward diffusion of colour from areas of more thermostable colour reaction. Nevertheless, in experienced hands and using a dilute solution of hydrogen peroxide, the loss of a general colour reaction could be used as a criterion of peroxidase destruction with both guaiacol and benzidine but this is not to be recommended. It may be remarked that with plant organs having relatively little differentiated tissue, such as potatoes, these difficulties do not apply, and that, in general, both of these indicators seem to be reasonably useful, especially guaiacol.

Lastly, attention may be directed to the results of recent storage tests on dehydrated beans. These are being carried out at this laboratory by Messrs Boyes, Ginsburg, and de Villiers. Tests have been made with 23 samples of beans involving 16 varieties. In all cases the beans were blanched for 6 minutes in a 0·22 per cent. solution of sodium sulphite, and, after dehydration, were stored at 80° F. in nitrogen in sealed cans. At the end of a year they were in excellent condition without trace of discoloration, " off " flavour or unpleasant odour. Beans blanched in sodium sulphite solutions (0·22, 0·29, and 0·58 per cent.) for one hour, however, still give a positive benzidine reaction. At the very least it is clear from these facts that the thermostable benzidine-hydrogen peroxide colour reaction of bean pods is irrelevant to adequacy of blanching.

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SUMMARY.

1. The general anatomy of the pod of *Phaseolus vulgaris* is described and illustrated.

2. Fresh bean pod tissue gave no colour reactions with either guaiacol or benzidine in the absence of hydrogen peroxide. The differentiated tissues developed a slight blue colour when scalded pod slices were immersed, without hydrogen peroxide, in benzidine solution for half an hour, but no colour was obtained with guaiacol solution.

3. On adding hydrogen peroxide all the tissues of fresh pods, except possibly the outer surface of the seed coat, gave a blue colour with benzidine and a reddish brown or brown colour with guaiacol.

4. The colour reactions obtained with fresh and blanched beans can be divided into the following groups :

(i) A general colour reaction of parenchymatous and differentiated tissues with both peroxidase indicators. This reaction was destroyed in 3-5 minutes at 100° C.

(ii) A relatively thermostable brown colour reaction of certain differentiated tissues with guaiacol and hydrogen peroxide.

(iii) A thermostable benzidine-hydrogen peroxide blue colour reaction of differentiated tissues. This reaction was obtained with bean slices that had been boiled in water for an hour.

5. The presence of traces of acetanilide in hydrogen peroxide solutions had no significant effect on the more thermostable colour reactions in bean pods when guaiacol and benzidine were used as peroxidase indicators.

6. The thermostable benzidine-hydrogen peroxide reaction was found to be located in the pericycle fibres, in the inner mesocarp (fibrous layer) and, less consistently, on the inner surface of the testa and in the xylem region (including the vessels) of the vascular bundles of the outer mesocarp, but excluding the suture vascular tissue. These effects were obtained in both young and old pods.

7. The relatively thermostable guaiacol-hydrogen peroxide reaction was shown by the same tissues as those which showed the thermostable benzidine-hydrogen peroxide reaction. In addition, however, the relatively thermostable guaiacol-hydrogen peroxide reaction was shown by the xylem vessels of the suture vascular bundles, the hypodermis and the epidermis.

8. The evidence presented indicates that the thermostable benzidine-hydrogen peroxide reaction is not due to the presence of a thermostable peroxidase ; and, in any case, this reaction is not related to adequacy of blanching. Thus, for bean pods, benzidine is to be regarded as an unsuitable peroxidase indicator.

9. Guaiacol when applied to bean pod tissues is, in general, also unsuitable for testing the adequacy of blanching, although for some lots it can be used.

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INVESTIGATIONS ON EGG-KILLING WASHES

III. THE OVICIDAL PROPERTIES OF CERTAIN ORGANIC THIOCYANATES

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THE existence of ovicidal properties among the organic thiocyanates was recorded in 1936 (Kearns and Martin, 1936) when successful results were given in tests of lauryl rhodanate (*n*-dodecyl thiocyanate). At a spray concentration of 0·4 per cent., this thiocyanate was toxic to eggs of *Aphis pomi* De Geer. (the Green Apple Aphis) and *Psylla mali* Schm. (the Apple Sucker), and it was shown that this property was unimpaired by application in petroleum oil solutions emulsified with soap or with the proprietary emulsifier Agral S.R.

Unfortunately, for economic reasons, this thiocyanate was not produced on a commercial scale in this country and it became necessary to examine other aliphatic thiocyanates. Kearns and Martin (1939) reported the results of field tests of the related β-butoxy-β'-thiocyanodiethyl ether (butyl carbitol thiocyanate), a substance produced in the United States of America under the name Lethane 410. Washes containing 0·3 per cent. of this thiocyanate and 3 per cent. of petroleum oil of the grade used in winter washes gave a high control of *Anuraphis padi* L. (the Leaf Curling Plum Aphis) on plums. Shaw and Steer (1939) included this and other thiocyanates in their laboratory and field trials and confirmed that both lauryl rhodanate and the carbitol thiocyanate were suitable components of winter washes. They also examined other thiocyanates, in particular those of the secondary alcohols, but found none of value. Among the primary alcohol thiocyanates, Kearns and Martin (1936) found that the cetyl derivative was much less ovicidal than lauryl rhodanate, in agreement with the conclusion of Bousquet *et al.* (1935) that there is, in this series, a peak of insecticidal activity at about the C_{12} member.

In the work here reported, the thiocyanates examined have been extended to include recent commercial introductions in the Lethane series; and the effects of compounding on ovicidal properties have been investigated against a wider range of insect eggs.

MATERIALS.

Dodecyl thiocyanate. Prepared from commercial lauryl alcohol, and fractionally distilled to give, as predominant constituent, *n*-dodecyl thiocyanate, $C_{12}H_{25}.SCN$. Commercially known as lauryl rhodanate, it is described in this paper as dodecyl thiocyanate.

Lethane 410. A commercial preparation stated to contain 75 per cent. of β-butoxy-β'-thiocyano-diethyl ether (*n*-butyl carbitol thiocyanate), $C_4H_9O.CH_2.CH_2.O.CH_2.CH_2.SCN$, and 25 per cent. kerosene.

Lethane 384 Special. Stated to contain 12·5 per cent. of butyl carbitol thiocyanate, 37·5 per cent. β-thiocyanoethyl laurate and 50 per cent. kerosene.

Lethane 60. Stated to contain 50 per cent. β-thiocyanoethyl laurate and 50 per cent. kerosene.

Lethane A 70. Stated to contain 90 per cent. $\beta\beta'$-dithiocyanodiethyl ether and 10 per cent. kerosene. In this compound the butoxy group of the thiocyanate present in Lethane 410 has been replaced by a second thiocyano group, $SCN.CH_2.CH_2O.CH_2.CH_2.SCN$.

Secondary alkyl thiocyanates. The samples of thiocyanates of the following secondary alcohols: hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl were from the same materials described by Shaw and Steer (1939). In addition, a batch sample of secondary (C_{10-12}) alcohol thiocyanates of distillation range 120-160° C at 3 mm. was included: samples supplied by courtesy of Technical Products Ltd.

γ-thiocyanopropyl phenyl ether, $C_6H_5O.CH_2.CH_2.CH_2SCN$ (Hartzell and Wilcoxon, 1934), and *trimethylene dithiocyanate*, $SCN.CH_2.CH_2.CH_2.SCN$ (Wilcoxon and Hartzell, 1935): samples supplied by courtesy of Imperial Chemical Industries Ltd.

White and brown mustard flours. Those used were kindly supplied by Dr. J. W. Corran, of Messrs. J. & J. Colman Ltd.

The remaining chemicals tested were from normal laboratory stock.

Methods of Spray Preparation.

The methods of emulsification used included the two solution oleic acid method (Martin, 1931) and milling to stock emulsions by means of sulphite lye (Kearns and Martin, 1937). The thiocyanate-oil mixtures were prepared either directly from stock emulsions compounded from thiocyanate-oil solution or indirectly by the mixture of appropriate dilution of separate thiocyanate-oil and oil stock emulsions.

Experimental Methods.

The methods used for the evaluation of ovicidal properties on the eggs of *Aphis pomi* De Geer. (the Green Apple Aphis), *Psylla mali* Schm. (the Apple Sucker), *Operophtera brumata* L. (the Winter Moth) and *Oligonychus ulmi* Koch. (the Fruit Tree Red Spider Mite) have already been described (Kearns *et al.*, 1937, Bennett *et al.*, 1946).

RESULTS AND DISCUSSION.

1. Tests on eggs of *Aphis pomi*.

In 1937 the supply of biological material permitted the use of replicates of batches each usually of well over 100 eggs. Reduction to percentage hatch in each replicate and a disregard of weighting in the calculation of mean hatch and its standard error were, therefore, permissible. The figures obtained are assembled in Table I. In other years the egg material available was insufficient for this treatment and smaller egg hatches were used per replicate. The mean percentage hatch was calculated from the total and the uniformity of the egg response was assessed by the calculation of the (Chi)^2 figure. These figures are given in Table II from which it will be seen

that there is a high degree of heterogeneity in the untreated eggs. This lack of uniformity in egg viability reappears in a few of those treatments which have little effect on egg hatch, but the (Chi)^2 figures are in general low enough to permit comparisons.

The efficiency of both dodecyl thiocyanate and of butyl carbitol thiocyanate as ovicides against *A. pomi* is confirmed by the results of Table I both when soap and sulphite lye are used as emulsifiers. The secondary alkyl thiocyanates fail, though there is a trend to greater ovicidal potency in the higher members.

From Table II it would appear that β-thiocyanoethyl laurate is inactive on eggs of *A. pomi*, even at 0·4 per cent.; the action of Lethane 384 Special is, therefore, due to the butyl carbitol thiocyanate present. The dithiocyanoethyl ether, on the other hand, is highly ovicidal. These differences in activity are unaffected by the presence of high boiling petroleum oil (Shell JD_2) of a grade conforming to the Ministry of Agriculture specification for petroleum oil winter washes.

TABLE I.

Action of thiocyanates on eggs of A. pomi.

	Conc. %	Total eggs.	Hatch %	n.	Conc. %	Total eggs.	Hatch %	n.
Applied March 9th: Emulsifier, 0·6% oleic acid + 0·1% NaOH.								
Dodecyl thiocyanate	0·4	1,603	0·1±0·07	8	0·3	2,856	2·3±2·17	8
Butyl carbitol thiocyanate	0·3	1,762	0·6±0·32	6	0·225	2,073	0·0±0·04	7
sec-(C_{10}-$_{12}$) alcohol thiocyanates	0·4	2,389	35·0±9·24	8	0·3	1,554	32·2±3·16	7
sec-hexyl thiocyanate	0·4	1,207	66·5±7·66	5	—	—	—	—
sec-heptyl thiocyanate	0·4	1,154	53·6±4·68	6	—	—	—	—
sec-octyl thiocyanate ..	0·4	1,435	30·4±7·95	6	—	—	—	—
sec-nonyl thiocyanate	0·4	1,335	54·6±6·16	8	0·3	976	27·0±8·09	6
sec-decyl thiocyanate	0·4	1,683	39·7±9·76	7	0·3	1,233	48·2±7·07	6
sec-undecyl thiocyanate	0·4	1,457	32·5±9·90	8	0·3	2,576	25·6±4·84	7
sec-dodecyl thiocyanate	0·4	3,005	8·1±2·80	7	0·3	648	11·4±2·82	5
Alkyl *iso*thiocyanate	0·4	1,623	24·8±8·92	10	0·3	2,838	57·4±7·11	7
Phenyl *iso*thiocyanate	0·4	3,437	14·4±2·20	8	—	—	—	—
tert-butyl thiocyanate	0·4	2,070	74·7±9·28	6	—	—	—	—
White mustard flour ..	1·0	3,126	72·9±7·53	7	—	—	—	—
Brown mustard flour	1·0	2,784	65·2±6·37	7	—	—	—	—
Tar oil (Grade A) ..	3·0	1,704	0·0±0·00	6	—	—	—	—
Emulsifier only ..	—	3,160	51·5±7·46	6	—	1,219	65·5±6·72	6
Applied March 9th: Emulsifier, 0·04% sulphite lye.								
Dodecyl thiocyanate ..	0·4	3,125	1·5±0·42	8	—	—	—	—
Butyl carbitol thiocyanate	0·3	2,941	1·4±0·43	7	—	—	—	—
sec-alkyl thiocyanates	0·4	1,051	13·9±4·72	7	—	—	—	—
Applied March 26th: Emulsifier, 0·6% oleic acid +0·1% NaOH.								
Dodecyl thiocyanate ..	0·4	2,228	0·03±0·03	6	0·3	3,505	0·0±0·00	7
Tar oil (Grade A) ..	3·0	1,990	0·0±0·00	8	—	—	—	—
Emulsifier only ..	—	2,518	50·5±6·60	6	—	—	—	—
Untreated	—	2,108	52·3±6·36	8	—	—	—	—

Table II.

Action of thiocyanates and petroleum oil on eggs of A. pomi.

	Thio-cyanate conc. %	Without oil.				With 5% oil.			
		Total eggs.	Hatch %	(Chi)²	n.	Total eggs.	Hatch %	(Chi)²	n.
Applied February 26th: Emulsifier, sulphite lye.									
Lethane Special 384	0·4*	883	0·0	—	5	1,674	0·0	—	5
,, ,, ...	0·225*	843	3·4	9·09	5	780	1·4	3·47	5
β-thiocyanoethyl laurate ..	0·4	602	81·4	30·00	5	1,875	81·5	5·71	5
,, ,, ...	0·225	710	72·3	9·28	4	958	82·3	5·15	5
ββ'-dithiocyanodiethyl ether ..	0·4	1,206	2·2	19·42	5	1,470	0·0	—	5
,, ,, ...	0·225	1,330	3·1	1·88	5	1,218	0·0	—	5
Emulsifier only	—	1,290	66·4	86·86	5	1,053	62·2	12·47	5
Untreated	—	3,432	76·2	616·24	19	—	—	—	—

* Thiocyanate conc. (calc.).

2. Tests on eggs of *P. mali*.

As the difficulty of obtaining suitable egg material prevented examination of the full range of thiocyanates, tests were limited to the comparison of those effective against *A. pomi* in the presence and absence of petroleum oil. The results of these tests, given in Table III, confirm the conclusion of preliminary work, previously reported (Kearns and Martin, 1936), that dodecyl thiocyanate, at 0·4 per cent., has the same order of ovicidal potency as 3 per cent. tar oil, the usual winter wash against this pest. This action is unimpaired by the addition of petroleum oil which appears to be antagonistic in action to that of butyl carbitol thiocyanate and β-thiocyanoethyl laurate.

3. Tests on eggs of *O. brumata*.

As Shaw and Steer (1939) found that dodecyl thiocyanate and butyl carbitol thiocyanate were deficient, at 0·4 per cent., in ovicidal properties to eggs of *O. brumata*, the tests of these two compounds were largely confined to thiocyanate-oil mixtures. As the eggs of this insect are effectively killed by the petroleum oil alone at 5 per cent. this concentration was used only for tests to determine whether or not the presence of the thiocyanate inhibits ovicidal action. The results given in Table IV show that both lauryl rhodanate and butyl carbitol thiocyanate are ineffective as ovicides on *O. brumata*; they do not reduce the potency of 5 per cent. oil sprays and do not permit the use of lower oil concentrations against *O. brumata*. The newer thiocyanates, β-thiocyanoethyl laurate and ββ'-dithiocyanodiethyl ether, are likewise ineffective at 0·225 per cent., but do not affect the potency of the 5 per cent. oil spray.

4. Tests on eggs of *O. ulmi*.

A disturbing feature met early in work on Fruit Tree Red Spider mite eggs was the high degree of heterogeneity in the egg material which is reflected in the frequent

TABLE III.

Action of thiocyanates on eggs of P. mali.

	Conc. %	Without oil.				With 5% petroleum oil.			
		Total eggs.	Hatch %	(Chi)²	n.	Total eggs.	Hatch %	(Chi)²	n.
Applied February 4th: Emulsifier, sulphite lye.									
Dodecyl thiocyanate	0·4	443	0·7	3·26	5	104	0·0	—	5
Lethane 384 Special	0·225*	186	11·3	9·72	6	391	63·4	46·62	6
β-thiocyanoethyl laurate ..	0·225	463	5·4	5·67	5	147	85·0	9·18	5
Emulsifier alone	—	567	76·7	15·72	4	311	65·0	8·32	5
Untreated	—	211	80·6	46·47	8	—	—	—	—
Tar oil (Grade A)	3·0	380	0·3	22·81	5	—	—	—	—

* Thiocyanate conc. (calc.).

high (Chi)² values recorded in Tables VI, VII, and VIII. Whilst this feature is doubtless associated with the difficulty and monotony of counting the small eggs and hatched mites, a task accomplished only by working for short spells to avoid eye strain, an attempt was made to determine whether there were avoidable biological reasons for differences in response between batches of eggs. Thus, in one year's trials, samples were separated into eggs laid on old wood and those laid on new wood, it being thought that the latter might yield more uniform results, either because of more uniform viability or of the more uniform wetting which the eggs might receive. The separate results for untreated twigs and for twigs treated with washes containing emulsifier only are given in Table V.

Combining the results for no treatment (Chi)² = 21·2, with six degrees of freedom; the results of the treatments with emulsifier solutions gives (Chi)² = 33·88, with seven degrees of freedom. The lowness of those figures and the similarity of the mean percentage hatch show that the heterogeneity is not associated with differences in viability for eggs laid on new and old wood respectively.

A differential response to ovicides of eggs laid on new and on old wood is not shown in the results assembled in Table VIII. Ten replicates were made in the treatments applied as dormant washes, five using new wood (A) and five using old wood (B). When the (Chi)² figure for the ten replicates was high, a separate calculation was made for the (A) and (B) woods. In eight cases the percentage hatch was greater on new wood, in five cases the older wood gave the greater hatch. Heterogeneity is not, therefore, associated with the use of twigs of different age.

Treatment differences are, however, sufficiently clear cut to emerge without further statistical analysis. Of the thiocyanates when used alone as ovicides, only dodecyl thiocyanate shows adequate toxicity to eggs of *O. ulmi*, whether applied at the dormant or delayed dormant period. Butyl carbitol thiocyanate has some effect but only when applied at the delayed dormant stage. The results with oil alone as the ovicide confirm previous experience that, even at 5 per cent., the hatch of eggs is not completely inhibited; with the addition of dodecyl thiocyanate, at 0·4 per cent., control is practically complete.

The results with other thiocyanates are not included in the Tables because

in no case was evidence of ovicidal properties obtained. These thiocyanates, which were applied in sodium oleate emulsions on February 26th at concentrations of 0·4 per cent. and 0·3 per cent., included *sec*-hexyl-, *sec*-heptyl-, *sec*-octyl-, *sec*-nonyl-, *sec*-decyl-, *sec*-undecyl-, *sec*-dodecyl thiocyanates, trimethylene dithiocyanate, γ-thiocyanopropyl phenyl ether, *tert*-butyl thiocyanate, butyl β-keto-γ-thiocyanate, methyl allyl thiocyanate, phenyl *iso*thiocyanate, allyl *iso*thiocyanate. Neither white nor brown mustard flours at 1 per cent. gave a lower hatch than the untreated controls.

TABLE IV.

Action of thiocyanate-oil combinations on eggs of O. brumata.

	Thiocyanate conc. %	3·0% oil.				2·0% oil.			
		Total eggs.	Hatch %	(Chi)²	n.	Total eggs.	Hatch %	(Chi)²	n.
Applied March 14th in sodium oleate.									
Dodecyl thiocyanate ..	0·00	530	5·7	36·0	5	—	—	—	—
,, ,, ...	0·03	696	7·8	2·0	5	859	6·4	2·9	4
,, ,, ...	0·05	925	7·0	20·7	5	—	—	—	—
,, ,, ...	0·1	1,194	5·4	13·0	5	349	17·8	5·5	2
,, ,, ...	0·4	898	5·8	7·6	5	611	6·2	2·2	3
Butyl carbitol thiocyanate ..	0·03	936	7·2	20·5	5	722	21·9	23·3	5
,, ,, ...	0·05	766	5·0	5·1	5	978	11·2	48·2	5
,, ,, ...	0·1	918	8·4	18·1	5	836	6·9	12·8	5
,, ,, ...	0·3	1,267	6·6	6·7	5	732	4·1	15·9	5
		No oil.				5·0% oil.			
Applied January 5th in sulphite lye.									
Dodecyl thiocyanate ..	0·4	118	52·5	21·54	5	114	0·0	—	5
Lethane 384 Special ..	0·225*	127	87·4	8·93	5	99	1·0	3·99	5
Nil	—	—	—	—	—	44	0·0	—	5
Untreated	—	314	94·3	7·92	5	—	—	—	—
Applied March 13th in sulphite lye.									
Dodecyl thiocyanate ..	0·4	98	22·4	7·07	5	336	0·0	—	5
Lethane 384 Special ..	0·225*	91	85·7	21·87	5	207	0·5	3·42	5
β-thiocyanoethyl laurate ..	0·225	223	72·2	29·41	5	210	0·0	—	5
$\beta\beta'$-dithiocyanodiethyl ether	0·225	133	50·4	32·33	5	90	0·0	—	5
Butyl carbitol thiocyanate ..	0·225	169	28·4	18·02	5	182	0·0	—	5
Nil	—	—	—	—	—	157	0·0	—	5
Untreated	—	440	92·7	9·48	5	—	—	—	—
Applied February 26th in sulphite lye.									
Lethane 384 Special ..	0·4*	375	42·1	23·27	5	—	—	—	—
,, ,, ...	0·225*	374	79·9	34·49	5	—	—	—	—
β-thiocyanoethyl laurate ..	0·4	200	47·0	9·50	5	—	—	—	—
,, ,, ...	0·225	298	84·2	109·12	5	—	—	—	—
$\beta\beta'$-dithiocyanodiethyl ether	0·4	404	52·0	32·40	5	206	0·0	—	5
,, ,, ...	0·225	381	68·0	40·91	5	300	0·0	—	5
Nil	—	—	—	—	—	290	0·0	—	5
Untreated	—	162	86·4	0·42	5	—	—	—	—

* Thiocyanate conc. (calc.).

TABLE V.

Viability of eggs of O. ulmi *on new and old wood.*

Treatment.	New wood. Hatched eggs.	New wood. Unhatched eggs.	Old wood. Hatched eggs.	Old wood. Unhatched eggs.	(Chi)²
Nil	536	32	737	43	0·0091
Nil	390	24	1,221	63	0·5108
Nil	1,510	79	1,249	68	0·0550
Nil	828	85	855	52	8·3624
Nil	589	49	2,616	241	0·3909
Nil	276	16	1,505	154	4·5153
Mean % hatch	93·5		92·9		
Sodium oleate	370	39	686	45	4·388
Sulphite lye	674	41	218	10	0·614
,, ,, ...	317	32	350	26	1·250
,, ,, ...	984	104	540	40	3·399
Bentonite	849	84	785	64	1·253
Sulphite lye	535	30	909	103	11·127
Cyclohexylamine dodecyl sulphate	966	73	728	50	0·25
Mean % hatch	92·1		92·6		

TABLE VI.

Action of thiocyanates on eggs of O. ulmi.

	Conc. %	Total eggs.	Hatch %	(Chi)²	n.
Applied February 28th, in sodium oleate.					
Dodecyl thiocyanate	0·42	2,657	3·0	67·59	16
,, ,, ...	0·2	906	36·8	32·23	5
Emulsifier alone	0·0	300	49·0	24·26	10
,, ,, ...	0·0	692	49·6	81·79	10
Applied March 31st, in sodium oleate.					
Dodecyl thiocyanate	0·42	1,002	1·3	5·09	8
Emulsifier alone	0·0	730	80·7	71·65	10
Applied February 26th, in sodium oleate.					
Dodecyl thiocyanate	0·4	302	39·4	19·0	5
,, ,, ...	0·3	371	89·5	6·9	5
Butyl carbitol thiocyanate	0·3	631	94·3	12·4	5
,, ,, ...	0·225	1,732	90·6	10·7	7
Emulsifier alone	0·0	1,300	85·9	149·83	5
Applied April 19th, in sodium oleate.					
Dodecyl thiocyanate	0·4	3,565	0·3	7·97	10
,, ,, ...	0·3	4,095	3·0	108·35	10
Butyl carbitol thiocyanate	0·3	3,068	45·1	247·60	10
,, ,, ...	0·225	3,298	55·3	44·54	10
sec C_{10-12} alcohol thiocyanates	0·4	2,856	55·3	101·61	9
,, ,, ...	0·3	2,988	77·0	24·58	10
Emulsifier alone	0·0	2,341	68·3	100·63	10

Table VII.

Action of thiocyanates on eggs of O. ulmi
(Dormant versus delayed dormant application.)

Thiocyanate.	Conc. %	Dormant, applied February 8th, 9th or 13th.				Delayed dormant, applied March 28th or 29th.			
		Total eggs.	Hatch %	$(Chi)^2$	n.	Total eggs.	Hatch %	$(Chi)^2$	n.
In sulphite lye emulsion.									
Dodecyl thiocyanate	0·5	2,109	0·0	—	10	659	0·1	2·24	5
" "	0·4	962	0·1	13·36	10	1,328	0·1	5·05	5
" "	0·225	1,604	0·2	17·59	10	2,192	2·8	40·37	5
Butyl carbitol thiocyanate ..	0·5	2,866	91·7	13·08	10	2,730	6·3	40·48	5
" "	0·4	2,863	89·7	34·00	10	1,972	22·4	89·07	5
" "	0·225	1,498	90·1	5·00	10	1,893	39·1	9·51	5
Lethane 384 Special	0·5*	897	88·2	4·57	10	3,660	84·9	20·20	5
" "	0·4*	3,112	94·6	15·23	10	2,694	89·4	8·45	5
" "	0·225*	1,546	77·4	263·70	10	1,928	91·8	8·34	5
β-thiocyanoethyl laurate	0·5	915	93·9	3·70	10	2,465	71·9	33·43	5
" "	0·4	3,702	93·2	43·18	10	2,417	82·7	44·40	5
" "	0·225	788	91·2	5·13	10	2,172	90·9	13·67	5
ββ′-dithiocyanodiethyl ether ..	0·5	—	—	—	—	1,385	89·5	16·90	5
" "	0·4	—	—	—	—	1,452	90·8	15·87	5
" "	0·225	—	—	—	—	1,494	88·4	9·79	5
Emulsifier alone	0·0	5,459	93·1	78·50	50	4,404	90·9	39·62	24
In sodium oleate emulsion.									
sec-dodecyl thiocyanate	0·4	1,098	90·6	7·44	10	551	77·3	13·78	5
" "	0·225	1,498	86·3	35·15	10	774	81·5	2·24	5
sec-C_{10-12} alcohol thiocyanates ..	0·4	1,263	91·2	7·93	10	1,374	77·4	5·64	5
" "	0·225	1,709	88·1	31·94	10	2,548	86·0	4·32	5
Emulsifier alone	—	1,140	92·6	83·35	10	1,339	78·5	3·52	5

* Thiocyanate conc. (calc.).

TABLE

Action of thiocyanate-oil

Thiocyanate.	Conc. %	With 1·0% oil. Total eggs.	Hatch %	(Chi)²	n.
Applied February 8th, 9th or 13th, in sulphite lye emulsion.					
Dodecyl thiocyanate ..	0·5	2,468	0·0	—	10
,, ,, ..	0·4	1,772	0·0	—	10
,, ,, ..	0·225	2,173	0·8	21·73	10
Butyl carbitol thiocyanate ..	0·5	1,835	78·3	72·35	10
,, ,, ..	0·4	2,534	41·2	89·44	10
,, ,, ..	0·225	2,024	73·0	52·94	10
Lethane 384 Special ..	0·5*	2,470	84·4	7·17	10
,, ,, ,, ..	0·4*	1,496	65·5	84·07	10
,, ,, ,, ..	0·225*	A: 706	29·3	10·40	5
		B: 797	87·3	9·61	5
β-thiocyanoethyl laurate ..	0·5	1,657	75·8	86·01	10
,, ,, ,, ..	0·4	1,260	86·5	59·16	10
,, ,, ,, ..	0·225	A: 211	90·0	11·98	5
		B: 221	78·7	25·28	5
Nil ..	—	2,042	73·2	33·72	10
Applied March 28th or 29th in sulphite lye emulsion.					
Dodecyl thiocyanate ..	0·4	1,222	0·0	—	5
Butyl carbitol thiocyanate ..	0·225	1,341	24·2	78·94	5
Lethane 384 Special ..	0·225*	2,794	79·2	16·72	5
β-thiocyanoethyl laurate ..	0·225	2,019	54·0	36·60	5
ββ′-dithiocyanodiethyl ether ..	0·225	1,390	15·6	50·92	5
Nil ..	—	3,755	33·5	38·49	5

* Thiocyanate conc. (calc.).

/III.

ombinations on eggs of O. ulmi.

3·0% oil.				5·0% oil.				7·5% oil.			
Total eggs.	Hatch %	(Chi)²	n.	Total eggs.	Hatch %	(Chi)²	n.	Total eggs.	Hatch %	(Chi)²	n.
1,178	0·0	—	10	2,272	0·0	—	10	3,201	0·0	—	10
1,419	0·0	—	10	1,773	0·0	—	10	1,060	0·0	—	10
2,229	0·4	116·7	10	691	0·0	—	10	1,944	0·0	—	10
A: 1,780	33·3	83·7	5	3,744	8·3	57·87	10	2,551	4·5	63·79	10
B: 1,131	47·6	19·61	5								
A: 463	23·1	184·56	5	1,800	12·1	39·23	10	2,505	2·5	23·48	10
B: 1,044	9·6	81·28	5								
A: 384	21·4	35·33	5	1,336	5·6	14·46	10	2,934	2·9	42·35	10
B: 1,898	12·0	178·19	5								
1,781	79·1	81·14	10	1,090	61·0	214·84	10	A: 917	9·3	75·61	5
								B: 1,080	0·9	4·83	5
1,890	28·5	343·28	10	2,643	14·3	184·35	10	3,422	5·1	93·34	10
927	44·8	252·00	10	1,346	7·2	9·86	10	1,663	7·7	24·33	10
1,854	27·1	121·31	10	A: 491	28·7	109·60	5	1,778	15·9	211·53	10
				B: 1,371	13·8	20·72	5				
A: 963	50·9	19·49	5	928	8·3	99·41	10	3,032	2·8	27·26	10
B: 364	79·4	5·32	5								
A: 453	40·0	3·72	5	A: 533	15·3	29·29	5	A: 786	3·2	3·94	5
B: 4,613	22·1	94·84	5	B: 1,170	7·4	3·41	5	B: 899	7·8	50·82	5
A: 353	10·5	18·14	5	A: 1,418	7·0	6·42	5	2,146	2·5	38·51	10
B: 410	23·5	4·44	5	B: 732	3·7	29·96	5				
1,467	0·1	3·60	5	2,327	0·1	15·53	5	697	0·4	2·24	5
1,646	1·7	12·87	5	1,277	0·3	23·25	5	1,757	0·3	5·81	5
1,925	7·8	21·51	5	1,716	5·5	12·73	5	1,488	0·5	16·57	5
2,264	1·2	8·77	5	1,321	0·0	—	5	2,085	2·5	3·71	5
2,028	7·0	63·46	5	1,501	4·3	23·37	5	2,305	1·1	2·21	5
1,065	10·6	25·44	5	1,071	5·0	3·67	5	1,986	0·7	44·17	5

SUMMARY.

In tests of the ovicidal action of certain organic thiocyanates on eggs of *Aphis pomi* De Geer (the Green Apple Aphis), *Psylla mali* Schm. (the Apple Sucker), *Operophtera brumata* L. (the Winter Moth) and *Oligonychus ulmi* Koch. (the Fruit Tree Red Spider Mite) it was found that :

(1) Dodecyl thiocyanate, butyl carbitol thiocyanate and $\beta\beta'$-dithiocyanodiethyl ether, in soap or sulphite lye emulsions containing 0·4 per cent. thiocyanate with or without 5 per cent. petroleum oil of winter wash grade, are effective against *A. pomi* eggs ; β-thiocyanoethyl laurate is ineffective at this concentration as also are the thiocyanates of secondary alcohols.

(2) In limited tests, dodecyl thiocyanate, at 0·4 per cent., with or without 5·0 per cent. petroleum oil, proved effective against eggs of *P. mali* when applied in sulphite lye emulsions ; both butyl carbitol thiocyanate and β-thiocyanoethyl laurate at 0·225 per cent. were ineffective when applied in 5·0 per cent. petroleum oil.

(3) Dodecyl thiocyanate (0·4 per cent.), butyl carbitol thiocyanate (0·4 per cent.), β-thiocyanoethyl laurate (0·225 per cent.) and $\beta\beta'$-dithiocyanodiethyl ether (0·225 per cent.) are ineffective against eggs of *O. brumata*, but do not reduce the ovicidal action of 5·0 per cent. petroleum oil emulsified with sulphite lye.

(4) Of the thiocyanates referred to in (1) only dodecyl thiocyanate at 0·4 per cent. is effective against eggs of *O. ulmi*, at which concentration it also augments the potency of 5·0 per cent. petroleum oil emulsions.

(5) Neither white nor brown mustard flour at 1·0 per cent. is an effective ovicide.

(6) A high variability shown in the hatching of eggs of *O. ulmi* and in the response of the eggs to ovicides was found to be unassociated with the age of the wood on which the eggs were laid.

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THE GROWTH OF THE PLANT IN RELATION TO THE INCIDENCE OF VIRUS INFECTION

A SURVEY OF THE LITERATURE

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Introduction.

From the earliest studies of plant virus diseases attempts have been made to correlate the incidence and intensity of disease with environmental factors. Thus, Mayer (1886), in the first important study of tobacco Mosaic, sought to trace some association between the nature of the soil, as revealed by chemical analysis, and the incidence of the disease in field-grown tobacco in Holland, but was led to conclude that " soils, even where the mosaic disease appears in them, are in an excellent state of fertility ". In some interesting footnotes, Mayer appended a collection of the views of growers concerning the cause of the disease. Of these, many appear strange to-day, but emphasis was placed by experienced growers on the association believed to exist between the disease and various environmental factors. Thus, lack of fertilizer, cold nights, frosty fogs, treatment at planting, planting in newly-turned ground, excessive dryness of the hotbeds and the use of pigeon manure had all variously been held responsible.

Very similar views have been held by potato growers (and by a few scientific investigators) as to the cause of heritable Leaf-roll (" Curl ") in the potato. A summary of some of these early views has been given by Orton (1914) and more recently by Pethybridge (1940). The importance attached by German investigators to an association between manuring and the incidence or intensity of Leaf-roll is particularly striking. Excess or deficiency of minerals and the use of manures of unbalanced composition have all been considered to be causally related to the disease, and work on this aspect of " degeneration " persisted up to 1935.

The empirical observation of experienced growers is still true to-day, namely that the presence of a virus disease is somehow dependent on the way in which a plant is grown. In the past many aspects of plant virus diseases have been studied, but apart from some German theorizing on potato degeneration, supported by little experimental evidence, few attempts have been made to enquire further into this belief of the practical man.

The relationship between incidence of virus disease and the environment.

The literature on plant virus diseases shows that many workers have noted some relation between environmental conditions and the incidence and intensity of disease symptoms in field or glasshouse. A summary of some field records is given in Table I.

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TABLE I.

Host.	Virus.	Factor associated with High incidence.	Factor associated with Low incidence.	Authority.
Tomato.	Tobacco Mosaic.	Impervious subsoil leading to water logging.	Favourable conditions for root development.	Bewley and Corbett (1927).
	,,	Excess potash in soil at planting and later.	Potash low but adequate for growth and fruiting.	Selman (1943a).
	,,	Topsoil of poor water-holding capacity.	Clay subsoil near surface.	Selman (1943b).
	,,	Unsteamed soil and cloudy weather.	Steamed soil and sunny season.	,, ,,
	Streak (Severe symptom of Tobacco Mosaic).	High humidity.	—	Poole (1924).
		Potash deficient in soil.	Potash adequate in soil.	Ainsworth (1932).
	Sugar beet Curly Top.	High evaporating power of atmosphere.	—	Shapovalov (1925).
	Big Bud.	High phosphate.	Gypsum reduced incidence.	Vertogradova (1941).
	,,	Use of wood ash dry.	Wood ash applied with water.	Vertogradova (1941).
	,,	Incidence related to moisture conditions of soil.		Trans. Conf. Plant Virus Diseases (1941).
Tobacco.	Tobacco Mosaic.	Coarse sandy soil.	Fine sands or sandy loam.	Curtis (1940).
		—	Favourable moisture conditions of the soil.	,, ,,
	,,	Pricked out seedlings.	Bed sown plants.	Curtis (1944).
	,,	Mixed fertilizer at 1 lb. per sq. yard.	Fertilizer without nitrogen.	,, ,,
	,,	Poor propagating soil.	Addition of properly made compost.	Howard (1940).
	,,	Badly growing plants and those roughly planted.	—	Roelofsen (1943).
	Tobacco Necrosis.	Apparently confined to plants under glass.		Smith (1937). Price (1938).
Sugar cane.	Mosaic.	Association with specific soil types in Cuba.		Sorenson (1939).
Cotton.	Leaf Curl.	Association with the growth of the plant as determined by the nitrogen status of the seedling plant.		Crowther (1941).

TABLE I (*continued*).

Host.	Virus.	Factor associated with High incidence.	Factor associated with Low incidence.	Authority.
Raspberry.	Viruses.	Acid soils. Dry seasons.	—	Jorstad (1928).
Potato.	Leaf-roll.	Drought conditions.	Shade conditions.	Wortley (1918).
	Mosaic.	—	High nitrogen and potash in early part of season.	Schulz and Folsom (1923).
	,,	Omission of potash.	Low nitrogen or omission of nitrogen.	Janssen (1929).
	Mosaic and Leaf-roll.	Excess stable manure and potash.	—	Pieper (1930).
	Pseudo-net necrosis and other viruses.	—	Moderate dressings of nitrogen with large amounts of potash.	Quanjer (1931).
	Viruses (various).	Excess nitrogen in soil, with early dry season followed by rain.	Dry weather and with addition of phosphates and potash.	Tereschenko (1941).
	Yellow Dwarf.	Varying amounts of fertilizer (5 : 10 : 5) had no significant effect on spread in the field.		Hansing (1943).
(Eigenheimer)	Mosaic.	Very light sand.	Heavy clay.	Hanken (1925).
(,,)	,,	Light sandy loam.	Heavy loam.	De Groene (1930).
(Duke of York).	Virus diseases.	No evidence that the extent and severity of these diseases differ on sand and clay.		Band (1928).
Lettuce.	Mosaic.	Dry weather during early part of growth period.	—	Ainsworth and Ogilvie (1939).
	Big Vein.	High soil moisture.	Low soil moisture.	Pryor (1944).
Sandal.	Spike.	High lime content of soil.	Lime normal.	Iyengar (1938).
Pineapple.	Yellow Spot.	—	Poorly growing areas of the field.	Carter (1932).
		Incidence conditioned by the growth and succulence of the plants.		
Sugar Beet.	Curly Top.	High light intensity. High temperature. High evaporation.	— — —	Carter (1929).
Peach.	Yellows and Little Peach.	Local conditions affect incidence of disease in stock of similar origin planted within ¼ mile of each other. Incidence not related to manuring.		Blake *et al* (1921).

Some workers have conducted experiments concerned with the influence of environment on the masking of symptoms and on the biology of insect vectors, but only to a limited extent on the susceptibility of the host plant. The incidence of virus disease in the field may also be related to the influence of environment on recovery (or loss of virus from the tissues), or to a direct effect on the viruses themselves, as for example, with soil-borne viruses. It is evident that any attempt to relate incidence data to the influence of the environment on the several relevant biological factors must necessarily be fraught with uncertainties, and only by experiments conducted under controlled conditions can a satisfactory analysis be made.

SYMPTOM SUPPRESSION.

Two conditions are frequently encountered in plants containing viruses: In the first the virus is present in high concentration but the plant exhibits no symptoms. This is referred to as " masking of symptoms ". Juice from such plants is usually highly infective. Of this condition two types have been recognized:

(*a*) Plants in which symptoms are suppressed as a result of environmental conditions, appropriate adjustment of environment leading to prompt appearance of symptoms. This may occur in the tomato as a result of drought or of nitrogen deficiency or, occasionally, of excessive nitrogen manuring with ammonium salts, or of high light intensities.

(*b*) Plants that are fully infective but never show symptoms. These have been termed " symptomless carriers ". No evidence has been found to suggest that this condition occurs in cultivated varieties of tomato. It should be noted, however, that Whitehead (1937) considered that there was no fundamental distinction between masking and carrying, and has put forward a theory according to which the plant cell is believed to exert varying degrees of dominance over the virus, depending on genetical and environmental factors.

In the second condition the virus is present in localized tissues only and at relatively low concentration. This condition is fairly common in the tomato. Kunkel (1939) found that tobacco Mosaic virus may remain dormant for long periods in tomato stem tissues. Fulton (1941) and Bawden (1943) report that Tobacco Mosaic virus may be present in high concentration in the roots and yet rarely move up into the tops. Experiments at Cheshunt (Selman, 1946) have shown that Yellow Mosaic virus may be present for some weeks in fruits and still be undetectable in the leafy shoots of rapidly growing plants. From these results it is possible to envisage a mechanism whereby careful culture may lead to the indefinite localization of tobacco Mosaic virus in the fruits or roots of the tomato.

It is certain that by judicious culture it is possible to cause virus disease symptoms in many crop plants to disappear, either through masking or recovery (Newton, 1923, Goss, 1924, Schweizer, 1930, Molenaar, 1936, Sorenson, 1939, Howard, 1942, Forbes and Mills, 1943, Dymond, 1944, Richards *et al.*, 1946). No instance of recovery by tomatoes from tobacco Mosaic virus infection has been observed at Cheshunt, but tomato plants infected with Spotted Wilt virus frequently develop shoots of healthy appearance which contain no detectable virus.

Attempts have been made at this Station (Selman, 1941, 1942a, 1943a, 1944, 1945c) to investigate the contention of growers that Mosaic in tomatoes is of little

consequence if the plants are properly grown. Many factors have been found to induce some degree of symptom masking, but within the limits of the experiments, plants that were virus-free have always produced not only a heavier crop than that from infected plants growing under similar conditions, but also one of superior quality. It is not to be supposed that this result is true under every condition; for example, growth under conditions of nitrogen or phosphate deficiency may not give a comparable result. Experiments by Rischkov and Smirnova (1939) with tomatoes in sand culture indicated that Mosaic infection caused no reduction in total dry weight of the plant when nitrate was applied, but led to a serious reduction when nitrate was withheld. However, data concerning all the nutrients applied and the stage of plant development were not given.

Animal vectors and the control of virus disease in the field.

The most important vector of tobacco Mosaic virus in the glasshouse tomato crop is Man. A suggestion that individual plants and particular crops differ in their capacity to resist infection introduced by Man's activities has been given earlier (Selman, 1943a and b).

Attempts have been made to control virus diseases in the field by destroying or otherwise eliminating insect vectors of the viruses. Bawden (1943, pp. 264-8) has summarized much of this work. Some of the difficulties in interpreting field data in this connection are evident from the report of Huckett (1945a) who dealt with the control of pea aphides in relation to Mosaic incidence. His conclusion (Huckett, 1945b) after an extensive investigation is of considerable interest: "For a number of years it was believed that this insect was a potent factor in the spread of mosaic disease, but all the experimental evidence in this investigation has failed to prove this relationship. In fact, the results tend to prove that mosaic may be severe even though the insect is controlled."

The control of the strawberry aphis is practicable under field conditions (Massee and Greenslade, 1941). Critical experiments dealing with the efficacy of the method in relation to the incidence of virus diseases have yet to be reported, although reports from growers are stated to be favourable.

It will be of interest to make reference to the control of virus diseases in the potato crop in which great significance has rightly been attached to the activities of insect vectors of certain of the viruses responsible for degeneration.

That aphides can and do transmit certain potato viruses is unquestioned. That a very marked measure of control has been obtained by importing "seed" potatoes raised in areas relatively free from aphides is also not in dispute, although the cost of such schemes to the farmer may be considerable. What may be questioned, however, is the assumption, generally inherent in studies of potato virus diseases, of a direct relation between the incidence of aphides and the incidence of disease. If it could be shown that the resistance of the plant to virus transmission by insects could be controlled, for example by manuring, some modifications in our present system of control might be found desirable.

Whitehead (1930) determined the correlation between loss in yield and the percentage of virus-diseased plants when potato stocks were allowed to degenerate naturally. High and significant correlation coefficients were found to exist between the number of plants affected with virus diseases and both the actual yield and the percentage loss in yield for two potato varieties. He stated: "With a correlation

of so high an order it is safe to conclude that factors other than virus diseases played only an insignificant part in the degeneration of potato stocks, or more accurately, that any such factor fluctuates with the degree of virus infection, and can therefore be ignored by the grower." It should be pointed out that such factors apparently do exist and in some crops can certainly not be ignored by the grower. An excellent illustration of this has been given by the comprehensive analysis of growth and disease incidence in cotton in the Sudan by Crowther (1941).

Studying the fluctuations in the annual yield of cotton, Crowther found a close correlation between the incidence of Leaf Curl virus disease and the total yield of cotton. Nitrogen analyses of the leaves revealed the existence of a significant correlation between this value, within two weeks of sowing, and the final yield of cotton, 6-7 months later. This association between potential vegetative vigour, as expressed by leaf nitrogen content, and the final yield, was established with statistical significance before Leaf Curl infection (or Blackarm bacterial disease) was observed on any plant in any year. It was concluded that a soil factor, with which the amount of nitrogen available for the crop is closely associated, was primarily responsible for the major yield fluctuations, and that the role of Leaf Curl was secondary. Cotton Leaf Curl virus is stated to be transmitted by the white fly *Bemisia gossypiperda*. It is not sap transmissible and is not carried in the soil (Kirkpatrick, 1931).

Whitehead *et al.* (1932) studied the aphis population of potatoes in relation to the incidence of potato virus diseases in various localities in North Wales. At certain centres there was little or no increase in virus diseases, but this could not be attributed to scarcity of aphides or to their non-infective condition. The authors stated that the maintenance of health in potato stocks is influenced not merely by the relative abundance of aphides but rather by the relation between the date of maximum infestation and *the stage of maturity of the foliage* (writer's italics). This conclusion would seem to be of considerable moment and should not be overlooked in assessing the long term conclusions of Whitehead (1943) to the effect that studies over a 15-year period indicate a definite correlation between the population of *Myzus persicae* and the degree of degeneration in the crops.

Field observations by Gregory (1943) might also be taken as indicating the importance of some factor other than insect transmission in the spread of viruses, regarded as aphis-transmitted. Factors controlling the incidence of winged forms, for example, may play some part in determining disease incidence ; but it is by no means certain that the resistance of the plant tissues to aphis-introduced viruses is unimportant.

Wortley (1918) noted that the spread of potato Leaf-roll was reduced by shade as compared with conditions of drought. Now conditions of high relative humidity such as prevail in the shade tend to inhibit the flight of *Myzus persicae*, as Maldwyn Davies (1935) showed ; but such conditions might equally prove to exert a favourable influence on the capacity of the potato plant to resist infection. It is to be regretted that few attempts seem to have been made to assess the relative importance of these and similar parallel effects induced by environmental factors. It may be noted that Watson (1936) considered that the influence of light on seasonal variations in infection of tobacco and Hyoscyamus with Hyoscyamus Virus III was on the plant rather than on the insect vector.

Stress has been laid on the importance of the insect vector rather than on the

condition of the host in studies of lettuce Mosaic by Ainsworth and Ogilvie (1939) who stated that : " Prevalence of mosaic disease in spring on winter hardy varieties of lettuces is usually correlated with dry weather, favourable to the multiplication of aphides, during the previous October and November when the plants were in the seedling stage." It may not be unreasonable to suggest that dry weather may tend to increase the susceptibility of the seedlings to aphis-borne virus infection, and frequency of aphides prove to be of secondary importance. The relation of watering and manuring to the susceptibility of glasshouse lettuce to mechanical inoculation with lettuce Mosaic virus has been studied by the present writer Selman (1945a). When the water supply was normal, heavy dressings of nitrogen led to increased susceptibility, but where more water was applied, the high nitrogen dressing was not associated with increased susceptibility to Mosaic. Heavy watering alone, as compared with normal watering, caused increased susceptibility. This result although not providing an answer to the observations of Ainsworth and Ogilvie, does suggest that useful results might accrue from a study of water supply and manuring in the field problem.

The question of the relative importance of the factors involved in field infection is a critical one, since control measures must be determined by the answer to it. With Mosaic infection in field lettuce, for example, should attention be devoted primarily to elimination of the aphis vectors or to irrigation and manuring of the seedlings ?

That plant growth does in fact sometimes influence the spread or incidence of insect-borne viruses in the potato is shown by the experiments and observations of Hanken (1925) and De Groene (1930) who found with the var. Eigenheimer that Mosaic diseases were more prevalent on sandy than on heavy soils. Band (1928), on the other hand, was unable to confirm Hanken's work, but used a different variety of potato.

That unbalanced manuring in some way influences the incidence or spread of potato viruses has been suggested by Schulz and Folsom (1923), Janssen (1929), Pieper (1930), Quanjer (1931) and Tereschenko (1941). Over-manuring of the potato may lead to yield reduction in the progeny especially in dry seasons (Hiltner and Lang, 1921), but although this and many environmental factors were discussed by Merkenschlager (1929) and others at Dahlem, no convincing data have been presented in favour of a total environment-induced degeneration in the potato. On the contrary, Brown and Blackman (1930) have clearly shown that degeneration of the potato does *not* occur in the absence of virus attack. It seems probable, however, that environmental factors do influence the susceptibility of the potato plant to virus infection as was suggested by Wartenberg *et al.* (1935), although the data upon which their statement was based seem open to another interpretation.

There is a parallel dearth of information concerning the relation which may exist between the nutrition of the plant and the incidence of the insect. Reference may be made to the instructive paper by Wittmer and Haseman (1945) on the effect of nitrogen nutrition in spinach and the incidence of Heliothrips. In general the plants selected for food by the thrips made less total growth, were higher in vitamin C, oxalate and phosphorus and lower in total nitrogen content. When supplies of calcium were increased at low nitrogen levels attack on the plants became less serious. Janssen (1929) noted that aphides developed most profusely on potatoes receiving no potash and least on those plants receiving no nitrogen. A significant

observation has been made by Watson (1942) to the effect that agricultural salt and nitrogenous fertilizers, which increased growth of sugar beet, also tended to depress the number of aphides (vectors of the Yellows virus) found on the plants.

There is clearly great need for more experimental work and attention may be directed to the fact that in a recent symposium on potato virus diseases held under the auspices of the Association of Applied Biology in 1942 (see Ann. Appl. Biol., **30**, May, 1943) no reference was made to the influence of soils and manuring on the incidence of disease. It may be suggested that the problem of virus disease in the potato crop might usefully be studied by experiments directed to the relation between the growth of the plant and its capacity to resist systemic infection by viruses introduced by aphides.

RESISTANCE OF THE PLANT TO VIRUS INFECTION.

That plants may sometimes possess the capacity to resist infection by viruses which normally invade their tissues readily, has been known for some years. Thus, for example, with increasing age of leaf or with any condition simulating old age there may be increased resistance to systemic infection (Bawden, 1943). Sheffield (1936) inoculated single hairs of leaves with the aid of a micropipette and concluded that many cells had the capacity to resist infection, since only 10 per cent. of inoculated hairs gave rise to systemic infection of the whole plant. Valleau and Diachun (1941) cite experiments with Mosaic-resistant tobaccos in which they were able to follow the spread of a bleaching strain of tobacco Mosaic virus by means of the chlorotic patterns developed in invaded leaves. It was concluded that resistance resulted from retarded multiplication of virus at the points of inoculation together with slow or complete failure of release of the virus into the vascular tissue for long distance spread. The mechanism which controlled virus release from local infections was unknown.

Failure of release of virus from local infections apparently ocurred in the experiments of Spencer (1937). He investigated the influence of N, P and K on the susceptibility of Turkish tobacco, grown in sand in 4 inch pots, to systemic infection with a Yellow Mosaic virus. When the tip of a leaf about halfway up the stem was rubbed with undiluted infective sap, it was found that variations in the amounts of nitrogen or potash applied were without effect in inducing any resistance, i.e. 100 per cent. of the plants developed systemic infection. With variations in phosphorus supply, all the inoculated plants showed the characteristic yellow primary lesions, but several failed to develop systemic symptoms. Plants receiving 60 mg. P/pot/ week for 5 weeks made the best growth, and in this group 19 per cent. of the plants failed to develop systemic symptoms. With higher applications of phosphorus, growth was inferior, but 22 per cent. of the plants failed to develop systemic symptoms. Phosphorus deficient plants became 100 per cent. infected. Böning (1928) concluded that the spread of virus from inoculated leaves of tobacco was retarded by deficiency of nitrogen.

Volk (1931) grew tomatoes in sand culture with varying amounts of N, P and K and investigated their susceptibility to Mosaic, but only small differences were observed. The incubation period was lengthened by deficiency of phosphorus, and the absence of nitrogen temporarily prevented the appearance of systemic symptoms (which developed when nitrogen was applied). With Streak (Mixed Virus ?) susceptibility was reduced by a shortage of phosphorus, in contrast to Spencer's result with Yellow Mosaic on tobacco.

Verplancke (1932) noted that addition of lime to the soil led to reduced susceptibility of Geranium to the Leaf Curl virus. With young tomato plants, Selman (1942b) reported that liming an acid soil reduced the susceptibility to infection with the Spotted Wilt virus when the leaves were wiped with infective sap, whereas sulphate of potash was without effect.

Using the carborundum-leaf-rubbing method of inoculation, Selman (1945a) studied the effect of N, P and water supply on the susceptibility of lettuce to lettuce Mosaic virus. Plants receiving high N plus medium P gave 100 per cent. infection, whereas those receiving low N, low P and a medium water supply gave only $12\frac{1}{2}$ per cent. infection. It was suggested that the available nitrogen content of the soil might be a factor of importance in determining susceptibility to infection. Brierley and Stuart (1946), using a similar inoculation method, worked on the susceptibility of the onion to Yellow Dwarf virus. Plants were grown at two levels of nitrogen manuring and it was found that both the percentage expression of symptoms and the actual percentage of infection were lower at the lower nitrogen level, both differences being highly significant.

Of the few environmental factors hitherto studied, it thus appears that the age of the leaf, together with the supply of nitrogen, phosphorus, lime and water may be of importance in determining resistance to virus infection. On *a priori* grounds it might be supposed that all the nutritional factors that contribute to leaf metabolism must play some part, however indirect, in controlling virus *multiplication* within the living cells. The study of the reactions of leaves of differing chemical constitution to inoculation with virus may thus be one line of attack on the problem, although it must not be overlooked that the *entry* of virus into the leaf tissues may be determined by morphological factors such as thickness of cuticle or presence and structure of leaf hairs.

At Cheshunt it has not been found practicable to conduct a full-scale series of experiments in sand culture to study the interaction of the several mineral nutrients on susceptibility to virus infection. Attempts have been made, however, to study the reactions of plants growing in soil under various manurial and soil moisture treatments, and these will be described in a further paper.

Conclusion.

From the foregoing it will be evident that the possibility of growing plants possessing the capacity to resist infection by viruses under field conditions has scarcely been recognized, despite the appreciation of this notion by growers and their advisers from times long before the etiology of virus diseases came to be investigated. The intensive study of cultural and manurial treatments designed to produce plants resistant to virus infection is strongly to be advocated. Such methods based on good growing procedure might do much to reduce the ever-increasing incidence of virus diseases in crop plants, which prophylactic measures and the constant importation of healthy plants from distant areas seem unable to check with any degree of permanence.

No mention has been made of the problem of breeding plants resistant to virus infection, a line of work which is clearly of great promise, and is, indeed, already being actively pursued in some quarters. Eventually it may be hoped that the genetical approach will be combined with environmental control to induce a health stability in our crop plants not hitherto achieved.

SUMMARY.

A survey of the literature pertaining to the relationship between incidence of virus disease and the environment is presented. The field problem is considered under three heads:

(*a*) Symptom suppression;
(*b*) Animal vectors and the control of virus disease in the field;
(*c*) The resistance of the plant to virus infection.

It is pointed out that little critical work has been done on the problem of growing plants capable of resisting virus infection under field conditions, or on the parallel problem of growing plants that are relatively immune from insect attack. Evidence is adduced which strongly indicates that studies on these lines may offer a fruitful approach to the problem of virus disease control in the field.

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CONTROL OF THE STRAWBERRY RHYNCHITES (*RHYNCHITES GERMANICUS* HERBST) WITH NOTES ON ITS BIOLOGY

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Introduction.

Formerly listed as *Rhynchites minutus* Herbst, this weevil was known to Theobald (1909) as the Minute Rhynchites. More recently the specific name has been changed to *germanicus* Herbst, with *minutus* as a synonym, and the common name to the Strawberry Rhynchites. Locally, in Kent, it is also called the Elephant Beetle, an apt but somewhat misleading term, because another pest of strawberry and cultivated Rubi, the Strawberry Blossom Weevil, *Anthonomus rubi* Herbst, is much more widely known by the same name.

The first report of damage (Anon., 1908) related to strawberry. A few years later Theobald (1911) mentioned the Minute Rhynchites causing damage to raspberries in Devon by girdling the tips of young canes and thus inducing branching. Nothing more was heard of this insect on fruit until Massee (1934) and Rolfe (1936) reported injury to strawberry plants in Kent and Sussex, respectively. By 1940 the weevil, though still local, appeared to have become well established in the Sutton Valence district of Kent (Massee, 1941), where an extensive strawberry growing area is situated. Five years later severe losses were being suffered by growers in the same area, especially in Kingswood, up to 75 per cent. of individual crops being lost. Between 1943 and 1946 the writer, during investigations primarily concerned with the aphis, *Pentatrichopus fragariae* Theobald, found the Strawberry Rhynchites widely distributed, though not a serious pest, in Hampshire, Worcestershire, Cambridgeshire and Essex. In addition to strawberry many cultivated forms of Rubus may also be attacked.

Occasional references occur in the literature to *Rhynchites aeneovirens* Mm.* attacking strawberry and raspberry. Whilst there seems no reason to doubt the authenticity of these records this species is usually associated with oak and has not been found attacking fruit during the last decade ; at present all evidence points to *R. germanicus* being solely responsible for the damage caused. In addition to *R. germanicus*, Balachowsky and Mesnil (1935) reported similar injury by *R. caeruleus* De G. in France. These authors illustrate a species of *Rhynchites* under the name *caeruleus* much more closely resembling *germanicus*, for scutellary striae are present on the elytra, a character not possessed by *caeruleus* ; this suggests the possibility of confusion regarding the species actually causing the damage reported on strawberry. The present writer has observed serious twig cutting by *R. caeruleus* on both hawthorn and apple in an Essex holding, but strawberries growing beneath the trees were attacked only by *R. germanicus*.

* A. Hoffmann (" Les Rhynchites de la faune française nuisibles à l'agriculture." *Ann. Épiphyt.*, **12**, 1946, 1-7) mentions *R. aeneovirens*, var. *fragariae* Gyll., as breeding on strawberry in France. This insect is found in England, but is not known to be associated with strawberry.

Recognition and economic importance.

Mention has already been made of one possible source of confusion since two pests of strawberry and cultivated Rubi, both weevils, possess the common name of Elephant Beetle ; further, they are very similar in size, measuring 3-4 mm. in length. Fortunately these insects, *R. germanicus* and *A. rubi*, can readily be distinguished by the following simple morphological characters :

R. germanicus.	*A. rubi.*
Antenna approximately equal-jointed, terminating in a club.	Antenna geniculate, basal joint equal to half total length.
Blue-green, metallic, with upright, fuscous pubescence.	Black, with sparse, depressed, ashy pubescence.

The type of damage caused by the respective insects is also easily recognized, *R. germanicus* girdles the stalks of young leaves and blossom trusses and the tips of young canes with a ring of punctures causing the entire portion above to shrivel and die, whereas *A. rubi* attacks only individual blossom buds, the female laying one egg in each and then piercing the blossom stalk with her rostrum so that the bud dies and eventually falls off.

The economic importance of *R. germanicus* is very closely related to the habits of the female at the time of oviposition. By girdling the stalks of blossom trusses great losses in crop may be sustained by strawberry growers, and the same type of injury to the young leaves (Plate I, Fig. 1) tends to reduce the vigour of the plants. Heavily infested plots have been observed in recent years where, by the end of May, many plants possessed only a peripheral ring of old leaves, the crowns being completely bare except for an accumulation of dead leaves and trusses cut off by the weevil as they appeared. The injury to stolons comes later, in June, July and August, when the weevil population is fast declining, and observations do not suggest that this seriously interferes with runner production. Damage to the blossoms of cultivated blackberry is not usually so severe, as only the terminal portion of the blossom spray is damaged ; nevertheless, the crop is reduced and, what is more important, that part of the spray which normally produces the earliest fruits is destroyed. Girdling of the tips of the current year's canes of cultivated forms of *Rubus* (Plate I, Fig. 2) results in branching which, besides increasing the difficulty of training, indirectly reduces the following crop ; for a weakly, branched cane is more susceptible to injury by frost during the winter and does not produce the same quality fruit as a sturdy, unbranched cane.

Life History.

Rolfe (1936) studied the life history of the weevil under insectary conditions. The present writer, by laboratory and field observations, has confirmed these findings and supplemented them by further details.

Adults overwinter in the soil and first appear on strawberry plants towards the end of March or early April, depending on the prevailing weather conditions. During the past two seasons the earliest weevils were observed on March 15th, 1945, after an exceptionally warm February and March, and on April 2nd, 1946. By the end of April the majority have left their winter quarters, but a few continue to appear until early May. Adults possess fully developed wings and will fly during hot,

sunny periods. If disturbed, however—and this species is very sensitive indeed—individuals do not seek safety in flight, but drop to the ground and feign death. Even a shadow falling on the plant is often sufficient to make them loose their hold.

Feeding begins soon after arrival on the strawberry plants. Early in April the weevils are mostly found tucked between the undersides of young leaves where they feed by eating small holes through them. On bright days some may be found sunning themselves on the upper surfaces of older leaves, whilst when the weather is dull many seek shelter in the crowns of the plants. By mid-April feeding of a more destructive nature begins, the weevils excavating small cavities in the petioles of partially opened leaves, many being made in a single stalk, with the result that the leaf soon wilts and finally dries up. Unless disturbed the weevil does not move far and will, in fact, often remain on a single plant until all the young leaves have been destroyed. Individual plants in lightly infested fields have been under observation for two or three weeks and one or two weevils always found on them whilst the food supply lasted, the neighbouring ones remaining unharmed. When no more young leaves or blossom trusses were available for food the attack was transferred to a neighbouring plant.

The thin-shelled egg is oval, translucent with a faint yellow tinge and measures $0{\cdot}7 \times 0{\cdot}5$ mm. In preparation for egg laying the female bores a hole in the stalk of a leaf or blossom truss, as when feeding, then proceeds to excavate a larger cavity inside. The number of eggs laid in a single stalk varies from one to four, and in addition there may be many other holes. The latter are either normal feeding punctures or abortive attempts at egg cavities. All the plant tissue removed from the stem is eaten. As soon as a stalk has received its full complement of eggs the female moves down it and makes a ring of punctures below the lowest egg; this interrupts the flow of sap sufficiently to cause rapid wilting and finally death. The dried-up leaflets, or the young blossom truss, may remain attached to the living portion of stalk for a considerable time, but eventually it breaks off and falls to the ground.

Eggs are first found about mid-April, in the stalks of partially opened leaves. A week later the earliest blossom trusses appear, and when these have stalks an inch in length they are also utilized for egg laying. Later, the tips of stolons are attacked, sometimes when only a few inches long, at other times not until they have extended to eighteen inches. Oviposition always takes place whilst the leaf, blossom truss or stolon is still young and tender; with blossom trusses it ceases as soon as the individual buds have become separated from one another and traces of petals are visible; in stolons only the terminal two inches are attacked.

Egg laying continues for a very long time, from mid-April until late August, with the peak period occurring during the latter half of April and the month of May. Besides strawberry, wild and cultivated blackberry, raspberry, loganberry and phenomenal berry are other host plants of this weevil. Much damage can be caused to cultivated blackberry during May by oviposition in the stems of blossom sprays when the buds are still closely clustered together. On this crop girdling usually results in the apical portion of the blossom cluster breaking off and hanging by a small piece of rind, closely resembling the twig-cutting injury to apple and hawthorn by a related species, *Rhynchites caeruleus* De G. At no time during these investigations have eggs

been found in the tips of young canes of Himalaya or Black Diamond blackberries, possibly because the cane is so vigorous ; on the other hand, eggs are often laid in leaf stalks near the tip. Observations also suggest that the weevils leave cultivated blackberry as soon as the blossoms separate from the tightly clustered stage, many migrating to wild blackberry where similar situations are chosen for oviposition, including the tips of the young canes. Other forms of *Rubus* suffer mainly from attacks to the tips of the current year's growth.

Two to three weeks after oviposition the egg hatches into a small, white, legless grub whose only food supply is the desiccated tissue of shoot or leaf girdled by the parent immediately after oviposition. When the egg has been laid in the tip of a shoot the larva passes its entire life burrowing in the dead tissue, gradually working towards the apex and devouring all except a thin epidermal layer which is left for protection. Larvae in leaf stalks and stems of blossom trusses behave in the same way, only here the stalk is smaller and the larva, after hollowing it, finds its way between the shrivelled leaflets or blossom buds where it continues to feed until mature. When fully fed the larva leaves the host tissue—which, for the greater part of the larval life is lying on the ground—and burrows into the soil where a cocoon is constructed by weaving a loose silken web to which soil particles are attached. Within this cell the larva soon changes to a pupa which, in turn, transforms to the adult stage after an interval of two or three weeks. The adult weevil remains in its earthen cell until the following spring when it emerges and starts feeding on one of the abovementioned host plants.

The period of development from oviposition to the adult stage varies considerably even under laboratory conditions, no doubt due in large measure to differences in the nutritive value of the food. The study of internal feeders presents special difficulties as dissection of the host is necessary to observe the egg and feeding larva, often resulting in sufficient disturbance to cause death. The following data have been obtained by making collections of wilted leaves, etc., in the field, keeping them in cages in the laboratory and inspecting samples at intervals.

On only two occasions were larvae found within fourteen days of oviposition, whilst all eggs had hatched by the end of the third week. Similarly, observations indicate that most larvae complete their development within six weeks, a few being found in the soil after five weeks and others remaining in the stalks until the eighth week. Larvae remain in the soil for a period of one to two weeks after leaving the host and before pupating, the adults appearing two to three weeks later. As an example, in freshly wilted leaves brought into the laboratory on May 3rd, 1946, only eggs were present on May 17th ; a sample taken a week later contained larvae only, and this condition continued until June 28th when the first larva was found in the soil. A pupa was present on July 5th, and by July 12th feeding had ceased, there being 16 pupae and 40 larvae in the soil. The first adult was observed a week later when all but two larvae had pupated, and by August 10th, with the exception of one larva, only adults were present. At the time of writing (5.12.46) this larva appears likely to overwinter. Further observations will be necessary to discover whether pupation takes place in the spring and the adult emerges at the normal time or whether the larva does occasionally lie over in the soil for a whole year, a fairly common phenomenon with some insects.

Under field conditions the period of development from egg to adult will probably be longer than that given above.

CONTROL.

In the past this pest has adequately been controlled by applications of a Derris dust (Massee, 1945). The following experiments were designed to test the effect of DDT and benzene hexachloride (of which the most toxic isomer is known as "Gammexane").

Laboratory Trials. Weevils were collected from the field on the day each trial began, and caged with young shoots of wild blackberry which they readily accepted as food. Those to be treated were first placed in a glass dish surrounded by a hurricane lamp glass through the top of which the dust was puffed. After 30 seconds they were transferred from the dish to a cage containing food. Each dust contained a carrier of gypsum and china clay. The DDT contained approximately 80 per cent. *p-p′* isomer and the benzene hexachloride 12 per cent. gamma isomer.

TABLE I.

Laboratory dusting trials against R. germanicus.

	No. of replicates.	Total no. of weevils.	No. dead after 5 days.	% mortality.
1% DDT	4	36	34	94
3% DDT	6	52	52	100
5% DDT	2	16	16	100
1% Benzene hexachloride ..	2	20	4	20
3% Benzene hexachloride ..	2	20	10	50
Control	5	46	2	4

Table I gives the results of these preliminary tests conducted between April 21st and May 12th, 1945. The mortality figures represent the numbers of weevils dead five days after treatment. It is of interest to note that identical results for the DDT treatments were obtained by adding together the totals listed as dead and paralysed on the day following treatment or subsequent days; after 24 hours no increase in the total casualties was noted, only the paralysed individuals gradually succumbed until, by the fifth day, all cages contained either apparently healthy or dead specimens. With benzene hexachloride the number paralysed was highest within 24 hours of treatment, then it decreased as some recovered. Another point of interest is the low concentration of DDT needed to kill this species.

Field Trials. Time did not permit of more than a demonstration of the efficiency of DDT in 1945. A small holding at Kingswood with half an acre of strawberries comprising the varieties Huxley, Lefebvre and Tardive was dusted on April 21st with 3 per cent. DDT on a gypsum-china clay base. The dust was applied at the rate of 24 lb. per acre with a hand operated machine, each row being dusted separately, and special attention being paid to getting the dust into the crowns of the plants. At the time of treatment a serious attack was already in progress, every plant showing damage to either leaves or blossom trusses with many eggs present. Observations were made on the weevil population following dusting; a few adults were found on the second day (April 23rd) but no more until May 8th when two were seen.

A slight, general infestation was built up during May but caused no injury of economic importance. There is no doubt that re-infestation occurred from neighbouring plots adjoining this holding, all of which were badly infested throughout this period, and separated only by low fences.

These results indicated that a 3 per cent. DDT dust could give protection from this pest for at least a fortnight.

In 1946 the same holding was used. It was conveniently divided by paths into four plots, two of $\frac{1}{8}$ acre, and one each of $\frac{1}{10}$ and $\frac{1}{13}$ acre, respectively. The treatments consisted of 3 per cent. DDT, 5 per cent. DDT and 5 per cent. benzene hexachloride dusts, each applied to a separate plot ; the fourth being left as a control. All the dusts, which had a gypsum-china clay base, were applied by a hand operated machine on the morning of April 20th under perfect weather conditions. Each row was dusted separately. Both DDT applications were at the rate of 20 lb. per acre, the 5 per cent. benzene hexachloride at 23 lb. per acre.

Weevils were first found on April 2nd, the numbers increasing up to the time of treatment ; by April 20th young wilted leaves were present on many plants and a few eggs were present. Apart from a slightly more severe attack on the plot receiving 5 per cent. DDT, the infestation was moderate and uniform. One blossom truss each was present on most of the Lefebvre plants, but very few had yet been damaged.

TABLE II.

Results of field dusting trial against R. germanicus, *1946*.

	May 3rd.			May 17th.		
	No. of plants inspected.	No. damaged.	%	No. of plants inspected.	No. damaged.	%
3% DDT	75	1	1	73	3	4
5% DDT	70	1	1	69	0	0
5% Benzene hexachloride	55	10	18	52	14	27
Control	52	11	21	49	14	29

As in the previous year observations on the adult population were continued after treatment, but the habits of the weevil made satisfactory counts impracticable. On plots receiving DDT no adults were found between April 22nd and May 18th, whereas some were present on the other two plots at each inspection. Results were assessed by inspecting plants for recent damage on May 3rd and May 17th. Starting at the corner of each plot single plants were inspected at three yard intervals in every third row. Any plants with leaves or blossom trusses in a wilting condition and with stalks girdled were listed as damaged.

Previously it has been stated that visual examination revealed no weevils on the DDT treated plots, whereas a study of the actual plants indicated injury to a few of them. That the method adopted for assessing results is more accurate than by counting weevils there can be no doubt, for the leaf and blossom truss injury is a permanent record of the weevil's presence whilst the insect itself may have escaped detection by hiding in the crown of the plant or falling to the ground.

The results, presented in Table II, show that both concentrations of DDT gave excellent control during the first fortnight ; even at the end of a month no reinfestation took place where 5 per cent. DDT had been used, and only slight damage was present on the 3 per cent. DDT plot. Benzene hexachloride, on the other hand, appeared to have very little effect, which is surprising considering that a 50 per cent. kill was obtained in laboratory trials with only a 3 per cent. dust. Either it failed to kill under field conditions, or an initial reduction in population was not maintained due to lack of residual toxicity, and further emergence of adults may have masked this reduction when records were taken a fortnight later ; the results suggest the latter. There is an additional possibility, namely that mortality in the laboratory trials may have been due in part to a fumigating action which would not occur in the field.

CONCLUSIONS.

Consideration of the above results shows that satisfactory control of the Strawberry Rhynchites can be achieved by dusting once with 3 per cent. DDT. Application should be delayed until the first flower trusses are appearing, as up to this time very little injury occurs and the weevil population is still being built up by emergence of fresh individuals from the soil. As this insect concentrates its attack on the young growth it is very important to ensure that an adequate amount of dust reaches the crown of each plant.

Against this pest on cultivated blackberry, the dust should be applied as soon as injury to the blossom trusses is noted, usually early in May.

Although a Derris dust was not included in the above trials it is well known that rotenone possesses only very slight residual toxicity as compared with DDT, and for this reason is likely to be superseded by DDT as a means of controlling the Strawberry Rhynchites, for biological observations have shown that weevils continue to emerge for at least a fortnight after the optimum time for treatment, a fact which would necessitate more than one application of a Derris dust if the attack was severe. With DDT a single treatment is effective.

The treated plots were also moderately infested with the Strawberry Aphis, *Pentatrichopus fragariae* Theobald, against which DDT appeared to have no effect. Routine population counts were taken throughout the spring and summer months and the population was found to vary in a normal manner. The Strawberry Blossom Weevil, *Anthonomus rubi* Herbst, was also present in small numbers and the 5 per cent. DDT and benzene hexachloride dusts appeared to reduce the numbers of this pest.

ACKNOWLEDGMENT.

The writer is greatly indebted to Mrs. Mercer, of Kingswood, who kindly permitted her strawberry beds to be used for the field trials.

SUMMARY.

Rhynchites germanicus Herbst overwinters as an adult in the pupal cocoon in the soil, emerging between mid-March and early May. On strawberry, eggs are laid from mid-April to late August in the stalks of young leaves and blossom trusses ; also

near the tips of stolons. Immediately after oviposition the adult girdles the stalk below the egg cavity, causing wilting and death of the terminal portion. The larva feeds by hollowing the dead tissue, and when fully fed burrows into the soil. Other host plants include wild and cultivated blackberry, raspberry, loganberry and phenomenal berry. The economic aspect of attack on these plants is discussed.

From the results of laboratory and field trials in 1945 and 1946 it is shown that on strawberries a single application of 3 per cent. or 5 per cent. DDT dust, having a gypsum-china clay base, at the rate of 20-25 lb. per acre, when the first blossom trusses appear, gave excellent control. 3 per cent. DDT gave protection for at least a fortnight, whilst no reinfestation was noted up to one month after dusting with 5 per cent. DDT. A 5 per cent. benzene hexachloride (" Gammexane ") dust with the same base, appeared to give only slight control.

Both DDT and benzene hexachloride at 5 per cent. reduced the numbers of *Anthonomus rubi* Herbst, but had no effect on the Strawberry Aphis, *Pentatrichopus fragariae* Theobald.

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PLATE I.

FIG. 1.

Centre: Two stolons and a young leaf of Royal Sovereign strawberry showing injury by *R. germanicus*. Left and right: undamaged stolon and young leaf respectively.

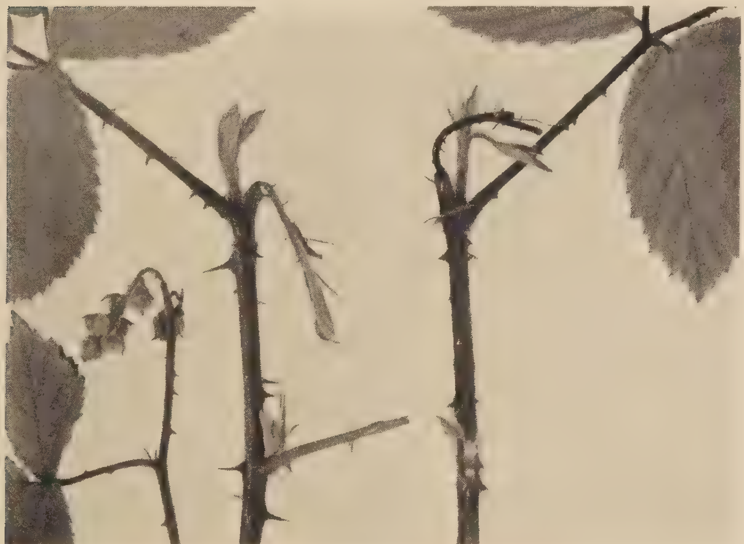

FIG. 2.

Centre and right: Two young canes of wild blackberry showing injury to shoot tips and resultant branching due to *R. germanicus*. Left: a damaged blossom truss.

RESISTANCE TO MOSAIC INFECTION IN THE TOMATO IN RELATION TO SOIL CONDITIONS

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Introduction.

From a survey of the literature (Selman, 1947) it was suggested that study of the factors leading to resistance by plants to infection by small amounts of virus might lead to the development of new methods of virus disease control in the field. Attempts have been made to relate soil conditions to disease incidence in the field, and of the factors studied, the moisture condition of the soil has been indicated as being of importance in this connection. Thus, Bewley and Corbett (1928) observed that the severity and extent of Mosaic in the tomato crop under glass may be increased by the presence of a high water table. They stated that breaking up the impervious subsoil followed by steam sterilization produced a marked improvement in the health of the crops. The relative effects of steaming and subsoil improvement were not assessed, however, and the mere destruction of infective particles in root débris might have accounted for the subsequent freedom from disease. Read (1934, 1935) studied the influence of waterlogged soils (and of poisons extracted from such soils) on the severity and incidence of Aucuba Mosaic in pot plants. Neither the soils, nor extracts prepared from them, induced Mosaic in healthy plants.

Later, Curtis (1940) studied the incidence of Mosaic in tobacco plants and found that favourable moisture conditions of the soil were associated with a low incidence of disease. There are other records (Selman, 1947) of association of virus diseases with certain soil types, some of which may have been related to soil water conditions.

Experiments with young plants in six-inch pots (Selman, 1946) have been reported, from which it has been concluded that soil conditions, as determined by the inclusion of various amounts of fertilizers, may affect the susceptibility of the tomato to infection with tobacco Mosaic virus. As a continuation of these preliminary studies, an investigation has now been made of the influence of two watering levels, together with several base fertilizer treatments, on the susceptibility of fruiting tomato plants growing in the ground, to infection with the Yellow Mosaic virus.

Experimental procedure.

Viruses of the tobacco Mosaic group may take some weeks to invade a plant systemically, even under summer conditions, and it was considered that only by obtaining even growth over a considerable period could resistance of crop plants be satisfactorily demonstrated. It has been found that unless special attention is given to them, pot-grown plants rarely equal in vigour similar plants growing in a border. For this reason, therefore, experiments were carried out on plants growing in the borders of a cucumber house, 70 ft. × 13 ft. With the limited facilities available, a formal study of manurial factors in relation to susceptibility could not

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be made, although many aspects of the problem can be studied only by culture in a homogeneous rooting medium with nutrients supplied as pure chemicals.

Method of inoculation.

Infection of tomato plants on a nursery with tobacco Mosaic virus commonly occurs following the entry of a small number of virus particles through slightly injured living cells. This may occur through root cells from infective débris in the soil, or from virus, present on the fingers or knives of workers, entering cells of the aerial shoot. An attempt was made to reproduce the latter mode of infection by wiping single leaflets of fruiting plants with a cotton wool pad soaked in diluted infective juice. The leaflets were wiped three times, using the fingers as support. No abrasive was used and the leaflets were not rinsed after wiping.

Manurial treatments.

Seven base fertilizer treatments were applied to plots before planting, and each treatment was applied to one plot (4 ft. × 8 ft. 3 in.) in each border of the house. The two borders received different amounts of water and have been designated Wet and Dry respectively. A plan of the layout for the first experiment (Summer : May-August) is shown in Fig. 1.

The choice of fertilizer treatments was made in relation to some of the earlier results obtained with plants grown in small pots. Conditions of mineral deficiency were not studied, since the soil in the borders was already in a high state of fertility as judged by the chemical analysis of soil from the untreated plot before starting the summer experiment. The treatments for this experiment are shown in Table 1.

Table I.

Manures applied May 6th.

Plot.	Dried blood. oz./sq. yd.	Superphosphate. oz./sq. yd.	Sulphate of potash. oz./sq. yd.	Dung. lb./sq. yd.
A	0	0	0	0
B	1	1	0	0
C	4	4	0	0
D	½	2	¼	0
E	1	4	½	0
F	4	16	2	0
G	4	16	2	28

The manures were raked into the topsoil about 7 days before planting and the borders were watered. In the autumn experiment the amount of potash applied to plot G was reduced from 2 to ½ oz. per sq. yd.

At this point it may be stated that from the data obtained from this part of the work no simple relation could be traced between the numbers of plants remaining virus-free and any of the conditions of soil or leaf which were investigated. Moreover, no clear correlations could be discovered between manurial treatments and virus infection. Suffice it is to say that there was an indication that excessive manuring

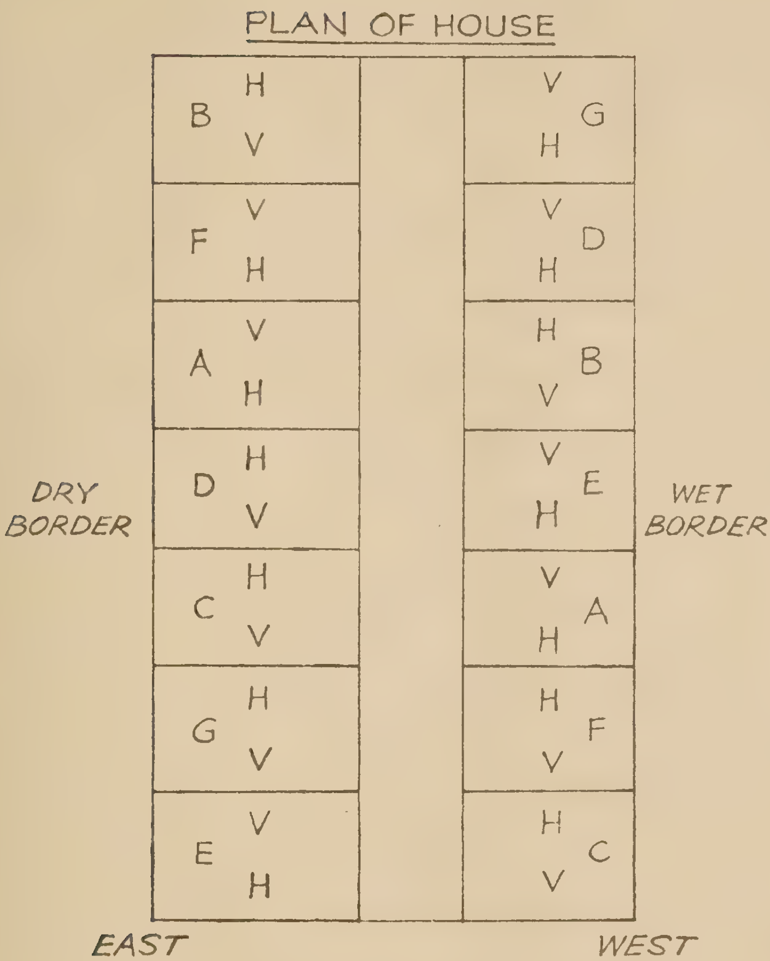

FIG 1.

Plan of Summer Experiment.

A-G = Different manurial treatments on plots 4 feet × 8 feet 3 inches.

H = Uninoculated controls (2 rows of 4 plants each).

V = Inoculated plants (2 rows of 4 plants each).

In the second experiment (Autumn. September-November) the East border received the wet treatment and the position of the H and V half plots was interchanged within each manurial plot. Additional fertilizer was applied before the Autumn experiment.

tended to lower resistance to infection. This seems to be in agreement with Curtis (1943-44) who found the incidence of Mosaic to be relatively low when nitrogen was omitted, but relatively high when a mixed fertilizer was applied. In view, therefore, of the difficulty of correctly interpreting the results obtained, details of the analyses made and of the effects due to the manurial treatments, will not be presented in this paper. Attention will mainly be devoted to the effects of soil moisture on the plants in the various plots.

Watering.

The procedure adopted in watering the plants in the two experiments is set out in Table II. All plants received light overhead damping during sunny weather.

Table II.

Summer experiment.

Date.	Dry border.	Wet border.
May 6th Fertilizer applied	Heavy damping.	Heavy damping.
,, 10th	Watering ½ min. per plot.	Watering 1½ mins. per plot.
,, 13th Plants set in borders	—	—
,, 13th-29th	1 watering of root ball after setting out.	4 heavy waterings over whole plot.
,, 29th Plants inoculated	—	—
,, 29th-July 29th	1 watering over whole plot (June 11th).	18 waterings over whole plot.

Autumn experiment.

Date.	Dry border.	Wet border.
Sept. 9th Fertilizer applied	Light watering over whole plot.	Light watering over whole plot.
,, 16th Plants set in borders	—	—
,, 16th-Oct. 4th	4 root ball waterings.	6 heavy waterings over whole plot.
Oct. 4th Plants inoculated	—	—
,, 4th-Nov. 20th	1 watering over whole plot (Oct. 25th).	7 waterings over whole plot.

Generally the plants on the dry borders were watered only when this became imperative to prevent wilting, whereas plants on the wet borders were thoroughly watered every three or four days.

Plant Material.

1. *Summer experiment.* Seeds of Potentate were sown on April 11th, and the seedlings were transferred to 3½-inch pots on the 25th. On May 13th, when plants had developed 6-7 leaves each, they were planted in the borders. There were 8 plants in each plot and on May 29th, when the plants were at the 13-14th leaf stage, one half of them were inoculated with Yellow Mosaic virus and the remainder were left as uninoculated controls.

2. *Autumn experiment.* Seeds of Potentate were sown on August 15th, the seedlings transferred to 3½-inch pots on August 29th and the plants set in the borders on September 16th, when at the 6 leaf stage. On October 4th the plants were inoculated as before.

INOCULATION.

The inoculum was prepared from leaves of White Burley tobacco, showing brilliant yellow mottling due to infection with Yellow Mosaic virus. Two and a half grams of tobacco leaves were macerated with water, filtered through cotton wool, and the extract made up to 250 ml. (Dilution $\times 100$). The terminal leaflet of the sixth leaf from the base of the plant was wiped three times with a cotton wool pad soaked in the diluted infective juice, which was tested for infectivity by wiping over leaves of *Nicotiana glutinosa* and found to contain virus. For the autumn experiment the inoculum was prepared from infected tomato leaves to dilution $\times 120$.

Records were kept of the appearance of symptoms in all the plants. As symptoms developed in them, the plants were removed from the house, care being taken to avoid contact with healthy plants. At the conclusion of the experiments, plants that had remained symptom-free were tested for virus; roots, youngest leaf and the inoculated leaf itself being tested by wiping juice from them over leaves of *N. glutinosa*.

In Table III are given the numbers of plants found to be virus-free at the conclusion of the experiments, 10 weeks after inoculation in the summer experiment and 7 weeks after inoculation in the autumn one. A few plants developed symptoms of Mild Tobacco Mosaic in the summer experiment. These were removed from the house and have not been included in the data presented below.

TABLE III.

Treatment.	Summer Experiment.		Autumn Experiment.	
	August 9th. No. of plants virus-free in Shoots.	Shoots and root.	November 20th. No. of plants virus-free in Shoots.	Shoots and root.
A Wet	2 out of 8	2 out of 8	2 out of 8	2 out of 8
Dry	4 ,, ,, 8	1 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8
B Wet	1 ,, ,, 6	0 ,, ,, 6	0 ,, ,, 8	0 ,, ,, 8
Dry	5 ,, ,, 8	4 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8
C Wet	2 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8
Dry	1 ,, ,, 8	0 ,, ,, 8	2 ,, ,, 8	1 ,, ,, 8
D Wet	1 ,, ,, 6	1 ,, ,, 6	0 ,, ,, 8	0 ,, ,, 8
Dry	0 ,, ,, 8	0 ,, ,, 8	4 ,, ,, 8	3 ,, ,, 8
E Wet	0 ,, ,, 6	0 ,, ,, 6	0 ,, ,, 8	0 ,, ,, 8
Dry	1 ,, ,, 7	1 ,, ,, 7	2 ,, ,, 8	1 ,, ,, 8
F Wet	1 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8
Dry	2 ,, ,, 8	0 ,, ,, 8	3 ,, ,, 8	3 ,, ,, 8
G Wet	0 ,, ,, 8	0 ,, ,, 8	1 ,, ,, 8	0 ,, ,, 8
Dry	2 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8	0 ,, ,, 8
Totals				
Wet	7 ,, ,, 50	3 ,, ,, 50	3 ,, ,, 56	2 ,, ,, 56
Dry	15 ,, ,, 55	6 ,, ,, 55	11 ,, ,, 56	8 ,, ,, 56

Where virus was present in the roots but not in the shoot, it was, with one exception, Tobacco Mosaic virus, arising presumably from root infection. No attempt at soil sterilization was made either immediately before or between the two experiments.

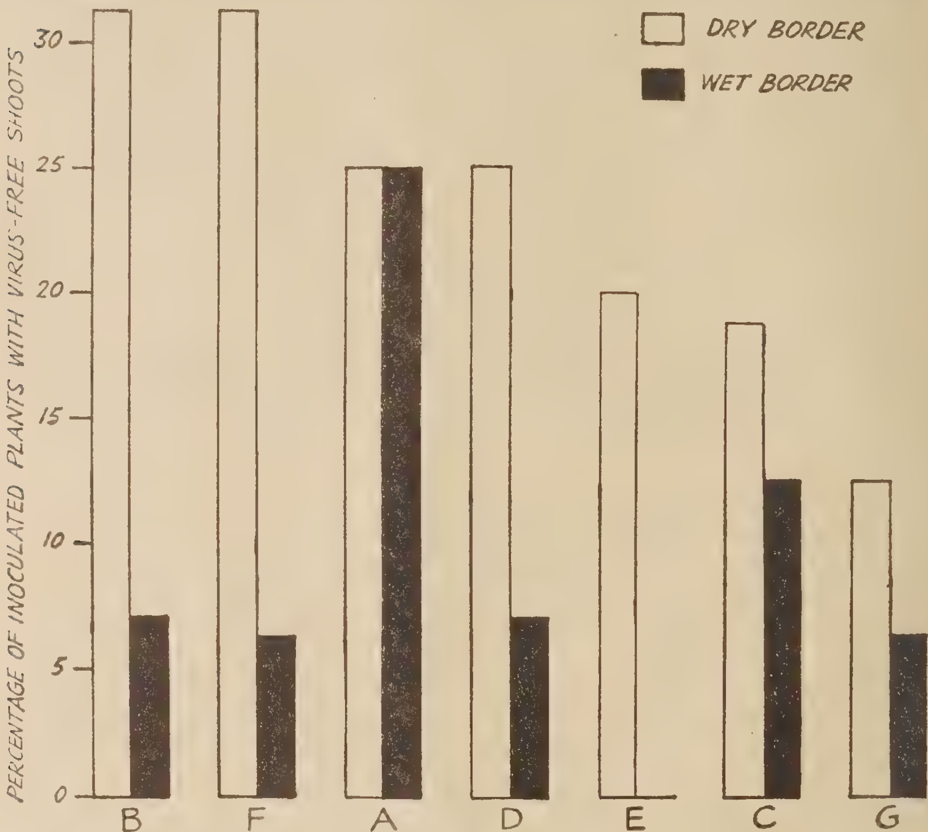

FIG. 2.

Illustrating the influence of wet and dry soils on the resistance of tomato plants to Yellow Mosaic Virus.

EFFECT OF WATER SUPPLY ON RESISTANCE TO MOSAIC INFECTION.

From the data assembled in Table III, and summarized diagrammatically in Fig. 2, there is evidence that over-watering tends to lower the resistance of inoculated plants to systemic infection. No information is available as to the specific soil factors which may be directly associated with these differences of reaction, but it may be of interest to record some further differences which were noted between plants growing in the wet and dry borders.

In the summer experiment, a count was made on July 18th of the number of plants showing yellowing of the lower leaves. This form of yellowing was similar

to that often attributed to magnesium deficiency. The count was made on both surviving control and inoculated (but symptom-free) plants. On the same plants, an estimate was made by two independent observers of the incidence of red spider mite which had been troublesome on some of the plots. The yield of fruit from 4 trusses was determined from the healthy (control) plants, and the percentage by weight of blotchy fruit was calculated. These data for plants of the wet and dry borders in the summer experiment are summarized in Table IV.

TABLE IV.

Treatment.	No. of plants with lower leaves yellowing.		Incidence of red spider mite.*		Oz. fruit per plant (4 trusses).		% blotchy fruit.	
	WET.	DRY.	WET.	DRY.	WET.	DRY.	WET.	DRY.
A	4 out of 7	1 out of 12	++	+	91	78	32	8
B	4 ,, ,, 6	1 ,, ,, 13	+++	++	84	60	41	7
C	3 ,, ,, 10	1 ,, ,, 7	—	—	94	97	19	19
D	2 ,, ,, 9	0 ,, ,, 6	++	—	76	92	12	15
E	3 ,, ,, 5	0 ,, ,, 9	++	++	100	89	30	21
F	4 ,, ,, 6	1 ,, ,, 8	++	+	79	81	31	14
G	3 ,, ,, 7	2 ,, ,, 8	++	—	81	100	31	15
Totals	23 ,, ,, 50	6 ,, ,, 63	Means	.. ..	86·5	85·3	28	14

* Red spider mite infestation : Very severe = + + +. Severe = + +. Slight = +. Absent = —.

Thus, adjudged by total fruit yield, plants of the wet border did not differ appreciably from those of the dry border. On the other hand the quality of the fruit was inferior on the wet border and leaf yellowing was much more severe. These observations suggest that soil conditions were definitely unfavourable for healthy growth on the wet border. It is also of interest to note that there appeared to be less red spider mite infestation on the leaves of plants in the dry border.

DISCUSSION.

From the evidence reported above, there is reason to suppose that some form of resistance to systemic infection by viruses of the Tobacco Mosaic type may occur in the tomato. There is little reason to suppose that commercially acceptable plants will be produced in the near future which are completely immune from these viruses, but it is considered that for practical purposes it should be possible to modify soil and climatic factors in such a way as to confer some degree of resistance upon tomato plants, so that where efforts to eliminate sources of infection are only partially effective (as so often occurs under commercial conditions) complete systemic infection of the aerial shoots may nevertheless be avoided.

From the limited data available, it may be suggested that excessive manuring with concentrated fertilizers and over-watering are to be avoided. Excessive manuring and over-watering are, as yet, ill-defined terms and must be qualified with reference to methods of culture and soil texture. From the data summarized in Fig. 2 it might appear that the deleterious effect of over-watering may be offset

by the omission of concentrated base fertilizers, for there was no difference in the percentage of plants remaining virus-free on the wet and dry borders where no additional fertilizers had been applied. One interpretation might be that the presence of a high salt concentration in the soil solution adversely affected the uptake of water by the roots. Indeed, on this soil, there was little reason to suppose that water-logging had occurred, at least for periods of more than a few minutes. Poor soil aeration might similarly retard water uptake, particularly in the tomato, whose roots are known to have a high oxygen requirement.

Practical measures which may be taken to reduce the incidence of Mosaic disease on tomato nurseries are common precepts of good growing procedure. Soil conditions and house temperature must be carefully studied and controlled accordingly. Consideration of the results of Went (1944) in conjunction with experience at Cheshunt both in virus control and in general tomato culture, indicate that night temperatures should be kept between 55° and 60° F. and day temperature should not exceed 80° F. for any length of time. When high day temperatures with bright sunshine are to be expected, shading of the glass is to be advised, for not only will this tend to lower the house temperature, but also help to conserve water, thereby rendering unnecessary heavy waterings which, in turn, necessitate frequent top-dressings of soluble fertilizers, all of which may lead to a deterioration in soil conditions.

In a healthy, well-grown tomato plant there may be several ways in which infective viruses may either fail to enter the tissues, fail to multiply within the cells or, having entered and multiplied, may become localized in fruits or roots (Selman, 1946). Failure to enter the cells may simply mean that the method of inoculation was such as to effect no damage to living cells. Thus in the present work, a resistant plant may have been one in which the cuticle or epidermal walls were especially thick or tough. Failure of the virus to multiply within the cells may involve more intimate considerations of cell metabolism. From the practical point of view the nature of the process may not be of great moment, but it would seem to merit further enquiry.

SUMMARY.

1. Experiments are reported in which the resistance of fruiting tomato plants to infection with the Yellow Mosaic virus has been studied by the inoculation of single leaflets with diluted infective sap.

2. Two successive experiments were carried out under glass in the same year with plants in the ground receiving seven different base fertilizer treatments together with two frequencies of watering.

3. Over-watering was found to decrease the resistance of plants to systemic invasion with this virus. Healthy plants similarly watered, showed more yellowing of the lower leaves and a greater proportion of blotchy fruit than did plants receiving less water.

4. The least effective treatment on the wet border resulted in 14 out of 14 inoculated plants becoming infected, whereas the best result was obtained in one treatment on the dry border in which 5 plants out of 16 failed to become infected.

5. Analyses were made of the soil at the time of planting, and of leaves comparable to those inoculated, at the time of inoculation. No simple relation could be

shown between resistance to infection and any single constituent of soil or leaf, and for this reason the details are not presented in this paper.

6. It is suggested that in hot, sunny weather systemic infection is likely to be minimized by shading the glass. Excessive watering under such conditions may be detrimental to the production of virus-free crops.

7. It is concluded that soil conditions are related to resistance of the plant to Mosaic infection. Of the many interrelated factors involved, there is evidence that under nursery conditions on a fertile soil, the soil water supply and the supply of concentrated fertilizers may be of some importance in this connection.

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MULTIPLE MINERAL DEFICIENCIES IN FRUIT TREES: INJECTION AS A FIRST AID TREATMENT

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INTRODUCTION.

A serious die-back of fruit trees that had for some years been causing alarm to growers in many parts of the country was found to be associated with a deficiency of potassium and of one or both of the trace elements iron and manganese (Roberts, 1945). The trouble was recorded in increasing extent from 1942 until 1945, mainly in apple trees, but in 1945 serious outbreaks were also observed in pears and plums.

The die-back was preceded and accompanied by an unhealthy appearance of the bark, a lack of extension growth and poor bud development, as well as by marginal leaf-scorch, which commonly accompanies potassium deficiency. More or less pronounced symptoms of manganese or iron deficiency, or of both, could in most instances be found in and around the die-back area.

Leaf analysis confirmed deficiencies of potassium and one or more of the two trace elements. In the majority of samples the iron content was sub-normal and calcium was present in abnormally large amounts, whereas most of the samples from trees affected by lime-induced chlorosis of the iron type, previously analysed, had contained abnormally large amounts of iron (presumably in a form useless to the plant) and abnormally large amounts of calcium.

Examination of the manurial histories of the areas under consideration revealed that during the war applications of potassic fertilizer had practically ceased, whereas heavy applications of various nitrogenous fertilizers and of lime had been given.

Potassic fertilizers were not likely to be available for fruit for some years to come and a further period might elapse after application before they exercised any marked effect. As branches, and even whole trees, had already died, the condition of the plantations over considerable areas was desperate.

These facts led to the conclusion that, under the circumstances, the possibility of supplying the deficient elements in the form of injections would merit investigation. It was realized that not enough potassium salts could be injected into the trunk to enable trees to bear a heavy crop of fruit, but the results of experiments already reported by Roach (1934a and b) raised the hope that a sufficient amount could be injected to prevent the death of many border-line trees. Experiments were carried out on this basis and the results were sufficiently encouraging to lead a number of growers to undertake tree-injection on a small commercial scale.

Leaf analyses, made during the growing season following the treatment, showed that the injection of potassium salts into the trunk of a potassium-deficient tree brought about an increase in the potassium content of the leaves. In addition, the results led to the quite unforeseen conclusion that injection of a trace element

into a tree deficient in both this element and potassium, seemed not only to cure the trace element deficiency, but also to alleviate the lack of potassium. The experiments on which these tentative conclusions are based are described in what follows.

PRELIMINARY WORK AT EAST MALLING IN 1944.

A few branches of apple trees, the leaves of which were affected by acute marginal scorch, were injected by one of us (W.O.R.) in June, 1944, with solid tablets containing salts of potassium and trace element by the method described by Roach and Roberts (1945) and Thompson (1945). The trace elements were injected at the rate recommended by Bennett (1931) and potassium sulphate at three times the rate recommended for manganese. There was an improvement in the appearance of the foliage of the injected branches in a few weeks. The following season the leaf symptoms did not appear and there was no die-back of the injected branches, in marked contrast to the untreated branches which were still affected by both leaf-scorch and die-back.

In a similar manner, one of two plum trees bearing leaves which, in 1944, showed acute potassium-deficiency symptoms, was injected in January, 1945, with potassium sulphate alone. In the summer of 1945 this tree was of a strikingly healthier appearance than the untreated one, having more and larger leaves of a much darker green colour.

DETERMINATION OF MAXIMUM DOSE OF POTASSIUM SALTS.

The two results just mentioned seemed to justify further work. In view of the fact that the amounts of potassium sulphate injected had been only a fraction of those necessary to produce normal crops, the first step appeared to be to determine the maximum dose of potassium salts that could be injected without damage to the tree. Separate branches were therefore injected with potassium sulphate and di-potassium hydrogen phosphate at 3, 6, 9 and 12 times the rate of dosage recommended by Bennett for manganese. The results are set out in Table I, and they suggest that Bramley's Seedling, and probably other varieties of apple, can be injected safely with 9 times the "manganese" dose of di-potassium hydrogen phosphate, and with 12 times the dose of potassium sulphate. It may be noted in passing that the two branches injected with the maximum safe dose of di-potassium hydrogen phosphate were singled out by unbiassed observers because each carried more blossom than the other branches of the trees.

EXPERIMENTAL WORK IN ESSEX, 1945.

Experimental injections of potassium salt plus trace element were made early in 1945 in four widely separated areas in Essex, where deficiencies had been diagnosed by leaf-analysis. The trace element was injected according to the amount recommended by Bennett and the potassium salt at three times that rate. It was unfortunate that the maximum safe dose was not known in time for it to be used in these experiments, which will now be described briefly in turn.

Locality 1. The die-back here was the worst observed in Essex in 1944. The symptoms preceding die-back were :

Chlorosis of the leaves at the tips of extension shoots, suggesting iron deficiency.
Interveinal chlorosis of spur leaves, suggesting manganese deficiency.
Marginal leaf scorch, suggesting potassium deficiency.

TABLE I.

Determination of maximum safe injection dose of potassium salts in Bramley's Seedling apple trees.

Date.	Dose.	Girth of branch (inches).	No. of holes.	Total no. tablets.	Degree of damage.	
					15.6.45.	30.4.46.
17.3.45	3 t S	15	5	15	—	—
	P	14½	5	15	—	—
11.5.45	3 t S	15½	5	20	—	—
	P	18	6	24	—	—
17.3.45	6 t S	12½	4	24	—	—
	P	16	5	30	—	—
11.5.45	6 t S	11	4	20	—	—
	P	12½	4	24	s	—
12.5.45	9 t S	11	5	30	—	—
	P	10	5	27	h	—
12.5.45	12 t S	14	7	56	s	—
	P	10	4	36	h	—

t=times trace element dose recommended by Bennett.
S=K_2SO_4.
P=K_2HPO_4.
—=no damage.
s=slight leaf scorch.
h—heavy leaf scorch.

The original leaf analyses in 1944 gave the following results :

	P. %	*K.*	*Ca.*	*Mg.*	*Mn.*	*B* (*p.p.m.*).	*Fe* (*p.p.m.*).
Best sample ..	0·23	v. low	high	normal	low	48	21
Worst sample..	0·21	,,	,,	,,	,,	21	17

Just outside the area occupied by trees affected with die-back it was found possible to select a number of closely similar trees suitable for experimental purposes which, up till then, had shown no die-back. The plan on page 83 indicates their position in relation to those in the die-back area and the injection treatment given to each tree. In addition to the trunk injections, two Bramley's Seedling trees were used for branch injection experiments. Injection was performed on March 7th, 1945.

EFFECT OF INJECTION ON APPEARANCE OF TREES.

In June, 1945, a number of independent observers all made identical observations viz. that the leaves of

all trees injected with Fe were of a deeper green colour than those of the untreated trees ;

all trees injected with K+Fe were of a deeper green colour than those of the trees injected with Fe.

The improvement was maintained in both sets of trees and became even more marked at the end of July. The effect of Mn injection was barely detectable.

PLAN OF TREES.

			↑ ← die-back area →			
•	•	•	COP (Fe)	•	DS (Mn)	•
•	B1*	•	COP (K+Mn)	•	DS (K)	•
CC (K+Mn)	•	•	COP (C1)	•	DS (C1)	•
CC (C)	•	•	COP (K)	•	DS (K+Fe)	•
CC (K+Fe)	•	•	COP (C2)	•	DS (C2)	•
CC (K)	•	•	COP (K+Fe)	•	DS (K+Mn)	B2*
•	•	•	COP (Mn)	•	DS (Fe)	•

Key. CC = Crimson Cox. B = Bramley's Seedling. COP = Cox's Orange Pippin. DS = D'Arcy Spice.
C, C1, C2 = untreated trees. * = trees used for branch injection. • = trees not used.

EFFECT OF TRUNK INJECTION ON THE MINERAL COMPOSITION OF THE LEAVES.

Leaf samples were taken periodically during the season and analysed. Each sample consisted of 20-50 leaves, that had just fully expanded, taken from extension shoots within reach of the ground. The results are given in Tables II to V.

It will be seen that the potassium content of the control trees C1 and C2 decreased in June, but rose again by the end of July.

TABLE II.

Effects of various trunk injections on the mineral composition of Cox's Orange Pippin leaves.

Treatment.	Dates of sampling. 14.5.45	16.6.45	30.7.45
	Percentage of potassium in leaves.		
C1*	1·4 } av.: 1·4	0·6 } av.: 0·6	1·1 } av.: 1·1
C2*	1·4	0·55	1·0
Mn	1·0	1·1	1·1
Fe	1·1	0·7	1·4
K	0·9	0·6	0·9
K+Mn	1·0	0·6	0·9
K+Fe	1·2	1·3	1·6
	Percentage of manganese in leaves.		
C1	0·002 } av.: 0·003	0·004 } av.: 0·004	0·004 } av.: 0·004
C2	0·003	0·004	0·004
Mn	0·012	0·016	0·008
Fe	0·003	0·004	0·004
K	0·003	0·004	0·003
K+Mn	0·009		0·020
K+Fe	0·002	0·003	0·004

* C1 and C2 = untreated trees.

The potassium content of the samples from the iron-injected trees also decreased in June, but it had increased by the end of July when it was 28 per cent. greater than that of the control. There was no similar decrease in the trees injected with potassium sulphate plus ferrous sulphate, the potassium content of which, by the end of July, was 50 per cent. greater than that of the control, and 73 per cent. greater than that of the potassium sulphate injected tree.

Comparisons of the iron injected tree with the control trees, and of the potassium plus iron injected tree with the tree injected with potassium alone, both suggest that the injection of iron led to the remarkable result of increasing the potassium content of the leaves, whereas injection of potassium sulphate apparently did not increase it. It has already been stated that the tree injected with iron and the one injected with iron plus potassium sulphate were improved in appearance, whereas the one injected with potassium sulphate alone was not.

The injection of manganese sulphate alone or combined with potassium sulphate caused a considerable increase in the manganese content of the leaves. The analysis of leaves for iron content showed no such consistent increase where iron had been injected alone or combined with potassium sulphate.

The analysis of leaves of D'Arcy Spice collected in July gave similar results as shown in Table III. The figures suggest that iron injection was again followed

TABLE III.

Effects of various trunk injections on the mineral composition of D'Arcy Spice apple leaves sampled in July, 1945.

Treatment.	K%		Mn%	
C1*	1·1	av.: 1·1	0·004	av.: 0·004
C2*	1·2		0·004	
Mn		1·5		0·019
Fe		1·8		0·005
K		1·7		0·004
K+Mn		1·7		0·018
K+Fe		1·9		0·006

* C1 and C2 = untreated trees.

by an increase in potassium content in the leaves and that manganese had a similar but less marked effect.

There were too few suitable Crimson Cox trees to perform the complete set of

TABLE IV.

Effects of various trunk injections on the mineral composition of Crimson Cox apple leaves sampled in July, 1945.

Treatment.	K%	Mn%
Control	1·0	0·004
K	1·2	0·004
K+Mn	0·9	0·009
K+Fe	1·1	0·004

injections, and those that were available bordered on the more normal part of the orchard. The results for samples taken in July will be seen in Table IV.

Only two (K and K+Fe) of the three potassium injections seemed to increase the potassium content of the leaves. There was no evidence that the iron or manganese injection had increased the potassium content of the leaves.

The results of injecting separate branches of two Bramley's Seedling apple trees will be seen in Table V. One of the trees, B1, bordered on the die-back area, the other, B2, was remote from it. (See Plan, p. 83.)

TABLE V.

Effects of different branch injections on the mineral composition of Bramley's Seedling apple leaves.

Treatment.	K%		Mn%	
	Tree B1.	Tree B2.	Tree B1.	Tree B2.
Control	0·7	2·9	0·004	0·004
Mn	0·9	2·2	0·027	0·015
Fe	1·0	2·2	0·002	0·005
K	0·9	2·2	0·003	0·006
K+Mn	1·0	2·1	0·017	0·013
K+Fe	1·1	3·1	0·003	0·004

The figures for the two trees differ markedly from each other. Those for the branches of tree B1, bordering on the die-back area, showed a considerable increase in potassium content as a result of ferrous sulphate injection and a correspondingly greater increase where this salt had been injected together with potassium sulphate. In tree B2, remote from the die-back area, however, injection produced no noticeable increase in potassium content over the control. Leaves of the uninjected branch of this tree contained almost five times as much potassium as those of the control branch on tree B1.

Locality 2. The experiments were carried out in about half an acre of 13-year-old Cox's Orange Pippin trees growing on a loose sandy soil and showing acute deficiency symptoms. The bark was of an unhealthy reddish-grey colour, whilst marginal leaf-scorch and typical interveinal manganese deficiency symptoms occurred, with here and there chlorosis of the tip leaves indicative of iron deficiency. The trees in the remainder of the orchard (some 18 acres) showed the symptoms less severely.

Analyses of leaf samples made in the summer of 1944 gave the following results :—

P. %	*K.*	*Ca.*	*Mg.*	*Mn.*	*B (p.p.m.).*	*Fe (p.p.m.).*
0·17	v. low	high	normal	low	25	67

The iron content though high when compared with that of the leaves from Locality 1, was below the 100 p.p.m. figure that is looked upon as normal.

The injections were made in March, 1945. In July, independent observers noted an improvement in foliage colour on the trees injected with K and Mn, while that of the trees injected with K+Fe showed some improvement later in the season. The results of analyses of leaves taken after the trees had been injected are shown

in Table VI. Here again, not only did injection of potassium sulphate lead to an increase in the potassium content of the leaves, but the injection of a deficient trace element (manganese) had an even greater effect. The effect of iron, though appreciable, was less than that of potassium itself. As already stated, manganese brought about an improvement in the appearance of the tree more quickly than iron did.

TABLE VI.

Effects of various trunk injections on the mineral composition of Cox's Orange Pippin apple leaves.

Treatment.	Percentage of K.		Percentage of Mn.	
	mid-June.	mid-July.	mid-June.	mid-July.
C1*	0·8 } av.: 0·8	0·8 } av.: 0·8	0·004 } av.: 0·004	0·0013 } av.: 0·0015
C2*	0·9	0·8	0·004	0·0017
Mn	0·7	1·1	0·016	0·011
Fe	0·9	1·0	0·004	0·005
K	1·0	1·0	0·003	0·003
K+Mn	1·0	1·2	0·011	0·009
K+Fe	0·7	1·0	0·003	0·003

* C1 and C2 = untreated trees.

Locality 3. The experiments were carried out on apple trees that showed severe symptoms of potassium deficiency, including a considerable amount of die-back, occupying an area of some 7 acres. Milder symptoms were noted in trees distributed over an area of 40 acres. Among the worst affected varieties were 22-year-old Cox's Orange Pippin and Worcester Pearmain. Early Victoria exhibited severe leaf-scorch but no die-back. The soil was boulder clay over gravel; the area having the worst affected trees was very poor light gravel.

Analyses of leaves from the badly affected Worcesters gave the following results:—

P. %	*K.*	*Ca.*	*Mg.*	*Mn.*	*B* (*p.p.m.*).	*Fe* (*p.p.m.*).
0·15	v. low	high	normal	border line	12	88

Compared with other cases described, the severe potassium deficiency here is not accompanied by so severe a deficiency of manganese or iron, although calcium is high, a fact which raises the question as to what extent the iron in the plant is in a form useless to it. The boron figure is, however, exceptionally low. Phosphorus is also low.

Injections were made in triplicate on March 9th, on a set of Worcester Pearmain trees. The treatments were K, K+P, Fe, Mn, K+Fe, K+Mn, K+P+Fe and K+P+Mn.

Independent observers noted in June and July an improvement in the trees injected with K+P. By late July the foliage of trees injected with iron was of a richer, darker green colour. It will be noted from the results given in Table VII that the amount of potassium steadily decreased in the control trees from June to

TABLE VII

Effect of various trunk injections on the mineral composition of Worcester Pearmain apple leaves.

Treatment.	K%			Mn%		
	June.	July.	August.	June.	July.	August.
Control		0·7 0·8 0·6	0·4 0·4 0·5		0·002 0·005 0·002	0·002 0·002 0·002
	0·9	Av.: 0·7	0·4	0·0021	0·003	0·002
Mn		0·9 0·9 1·0	0·5 0·7 0·5		0·014 0·010 0·009	0·029 0·014 0·011
	1·2	Av.: 1·0	0·6	0·010	0·010	0·021
Fe		1·0 1·0 0·8	1·0 1·1 1·0		0·004 0·003 0·003	0·002 0·004 0·004
	0·6	Av.: 0·9	1·0	0·0024	0·003	0·003
K		1·1 1·2 0·8	0·8 0·8 0·8		0·002 0·005 0·002	0·002 0·003 0·003
	1·0	Av.: 1·0	0·8	0·0022	0·003	0·002
K+Mn		0·9 0·6 0·9	0·6 0·7 0·6		0·11 0·005 0·01	0·02 0·01 0·03
	0·8	Av.: 0·8	0·6	0·008	0·009	0·02
K+Fe		0·7 0·7 0·8	0·5 0·9 0·5		0·004 0·003 0·002	0·002 0·004 0·002
	0·7	Av.: 0·7	0·6	0·0017	0·003	0·002
K+P		0·8 0·9 1·1	0·7 0·8 0·8		0·002 0·002 0·004	0·002 0·003 0·002
	1·4	Av.: 0·9	0·8	0·0034	0·003	0·003
K+P+Mn		0·6 1·0 0·7	0·7 0·4 0·6		0·012 0·010 0·006	0·015 0·014 0·017
	0·9	Av.: 0·8	0·6	0·006	0·01	0·014
K+P+Fe		0·8 0·8	0·7 0·5 0·4		0·002 0·002	0·002 0·002 0·002
	1·0	Av.: 0·8	0·5	0·0022	0·002	0·002

K = K_2SO_4 K+P = K_2HPO_4

August. The same tendency is also apparent in most of the treatments; it is, however, obvious that a marked increase in K content took place as the result of potassium injection both in the form of sulphate and phosphate. The phosphate seems to have produced a very striking improvement at an early date, but the increase in potassium content was more durable after potassium sulphate injections. The most striking results were obtained from the injections of ferrous sulphate alone. These injections were followed by a steady rise in the potassium level, culminating in an average content of 1·0 per cent. in August, which is $2\frac{1}{4}$ times the level of that of the control trees. It is interesting to note the poor results obtained from the K+P+Mn and K+P+Fe combinations. Ferrous sulphate alone, however, seems to have increased the amount of potassium in a spectacular fashion by August and, again, this was reflected in the darker green foliage. The injection of potassium phosphate did not raise the amount of phosphorus in the leaves above that in the control in June, July or August, and the iron and boron figures were very variable.

Locality 4. Severe leaf-scorch was apparent in 1944 in young Cox's Orange Pippin trees* distributed over an area of 5 acres.

This orchard had received no potash during 1939, 1940 and 1941; but 1 cwt. of muriate was applied per acre in 1942; 12 cwt. of flue dust (19 per cent. K_2O) in 1943 and 5 cwt. of vegetable ash (34 per cent. K_2O) in 1944.

The soil analysis, kindly supplied by the Chemistry Department of the Essex Institute of Agriculture, for the affected area was:

> pH = 6·64;
> lime requirement, low; phosphate requirement, very low; chalk reserve, very small.

A sample of leaves taken too late in the season (September 27th, 1944) to be regarded as a completely reliable indication of mineral status, gave the following analysis:—

P. %	*K.*	*Ca.*	*Mg.*	*Mn.*	*B (p.p.m.).*	*Fe (p.p.m.).*
0·20	border line	normal	normal	low	40	100

Trees were injected in March, 1945, in quadruplicate with K_2SO_4; $K_2SO_4+FeSO_4$, $K_2SO_4+MnSO_4$ and K_2HPO_4 respectively.

A composite sample of leaves from each lot of similarly injected trees was taken in mid-June with two control samples. A further sample, from each tree, was taken in mid-August. The results of these analyses are given below in Table VIII. There was no visible effect of the injection on the trees during the season following the injection.

The figures in Table VIII show that injections of potassium sulphate alone, or in combination with manganese or ferrous sulphate, increased the potassium content as compared with the control trees in June and August. The greatest increase arose from the injection of potassium sulphate plus ferrous sulphate. The potassium

* Interplanted with James Grieve pollinators which were remarkably free from deficiency symptoms. The outstanding resistance of this variety to potassium deficiency was confirmed by observations carried out in different parts of Essex. It may be due largely to rootstock influence. It should be stated that nearly all Grieves in Essex are grown on a crab rootstock, whereas Cox's Orange Pippin and Worcesters are almost invariably worked on Malling II.

phosphate injections caused a big increase in potassium content of the leaves by June, but this was not maintained into August. The injection of manganese sulphate led to a consistent increase in manganese content, otherwise the manganese content of the leaves appeared highly variable.

TABLE VIII.

Effects of various injections on the mineral composition of Cox's Orange Pippin apple leaves.

Treatment.	Percentage of K.		Percentage of Mn.	
	mid-June.	mid-August.	mid-June.	mid-August.
Control	0·4	0·4	0·004	0·004
	0·5	0·4	0·004	0·004
		0·5		0·002
		0·5		0·003
	Av. : 0·45	0·45	0·004	0·003
K_2SO_4		0·5		0·003
		0·8		0·004
		0·5		0·017
		0·6		0·003
	0·6	Av. : 0·6	0·004	0·007
K + Fe		0·7		0·004
		0·8		0·02*
		0·9		0·004
		0·6		0·004
	0·8	Av. : 0·8	0·005	0·007
K + Mn		0·4		0·02*
		0·9		0·02*
		0·8		0·02*
		0·5		0·02*
	0·5	Av. : 0·6	0·02	0·02*
K + P		0·5		0·004
		0·6		0·02*
		0·4		0·02*
		0·4		0·04
	0·8	Av. : 0·5	0·004	0·010

* The highest manganese content of all the standards used for comparison in the analytical method corresponded to approximately 0·02 per cent. of manganese. The true values usually exceeded this figure.

DISCUSSION.

Attention should be drawn again to the extreme variability of the trees used for the various experiments. In all the plantations mentioned the trees showing the severest symptoms occurred in patches, and in this respect the trees were far from

ideal for experimental purposes. But mineral deficiencies frequently affect trees in different parts of the same plantation to very differing degrees, and it is essential to develop methods for dealing with such material. In each locality groups of trees as comparable as possible were selected and the treatments were randomized amongst them. It is not surprising therefore that there are a number of irregularities in the analytical figures ; but they do seem to justify a few, at least tentative, deductions.

The effects on the potassium content of the leaf of injecting potassium and either manganese or iron respectively into trees deficient in both.

In Table IX have been collected the potassium content figures for all the experiments in which there was a definite improvement in the appearance of the foliage as a result of the injection.

Table IX.

Potassium contents of leaves of trees injected with manganese or ferrous sulphate, with or without potassium sulphate, arranged to show the effect of the trace element and of the potassium injection respectively.

	Mn.	Nil.	Fe.
Nil	1·1	1·1	1·4
K	0·9	0·9	1·6
Nil	1·5	1·1	1·8
K	1·7	1·7	1·9
Nil	—	1·0	—
K	0·9	1·2	1·1
Nil	0·9	0·7	1·0
K	1·0	0·9	1·1
Nil	1·1	0·8	1·0
K	1·2	1·0	1·0
Nil	0·6	0·4	1·0
K	0·6	0·8	0·6
Nil	—	0·5	—
K	0·6	0·6	0·8

It will be seen that in 12 out of the 17 possible comparisons potassium injection appears to have increased the potassium content, and in 9 out of the 12 possible comparisons iron injection appears to have increased the potassium content ; manganese in 6 out of 12. The fact that iron has given better results than manganese would seem to agree generally with our experience in Essex.

The average effect of the potassium injections was to increase the potassium content from 1·00 to 1·14, i.e. a 14 per cent. increase, whereas the effect of the trace element injections was to increase the potassium content from 0·95 to 1·14, i.e. a 21 per cent. increase.

ACKNOWLEDGMENTS.

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The writers wish to express their thanks to Dr. R. G. Hatton, Director of East Malling Research Station, to Mr. O. G. Dorey (who, whilst this work was in progress, was chief Horticultural Officer, Essex W.A.E.C.), and to Dr. W. A. Roach, Head of the Biochemistry Section, for their interest and encouragement ; also to Mrs. C. King, Miss B. Kidwell, and Miss D. Martin for carrying out the analyses.

SUMMARY.

Solid injection was used experimentally as a first-aid treatment to combat a serious and widespread attack of die-back in apple trees due to multiple deficiency of potassium and one or more of the trace elements manganese and iron. Experimental injections of the necessary nutrients into apple trees brought about an improvement in foliage colour and an increase in the potassium content of the leaves. Injection of the deficient trace element alone seemed to bring about an even greater increase in the potassium content of the leaves, and the injection of a deficient trace element plus a potassium salt led to a greater increase in potassium content than that following the injection of a potassium salt alone. Injections of manganese salts alone or combined with a potassium salt caused an increase in the manganese content of the leaf.

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EXTENDING THE STORAGE LIFE OF THE VICTORIA PLUM

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THE chief difficulty that has to be overcome in storing plums at low temperature is the occurrence of the physiological disorders grouped under the term "internal breakdown". It is well known that after a longer or shorter period of exposure to low temperature, according to variety and degree of temperature, the ripening of the fruit becomes abnormal (1). The symptomatology of these disorders has already been described (2, 3, 4), and some attempts have been made to overcome their occurrence by manipulating the level of storage temperature.

The two main approaches to this problem have been those of conditioning the fruit by exposure to a higher temperature, either before or after low temperature storage. Delayed storage, which has been reported to have a pronounced effect in the control of woolliness in Peregrine peaches in South Africa (5), does not seem to have been very efficient in controlling internal breakdown in Santa Rosa plums (6). Kelsey plums are reported (7) to have reacted favourably to delayed storage, the amount of internal breakdown being reduced ; but there was not always the accompaniment of an improved ability to ripen after storage. More recently, it has been shown in the U.S.A. (8) that partial ripening of Italian Prunes by exposure to a temperature of 65° F., before storage at 31° F., has been effective in avoiding breakdown and promoting normal ripening. A combination of acetylene treatment for 24 hours followed by delayed storage at a temperature of 65° F. up to a period of 8 days, duration, while reducing internal breakdown in South African Santa Rosa plums stored at 34° F., had an adverse effect in accentuating a condition described as "abnormal softening" (9).

Exposure of the fruit to a temperature of 65° F., after refrigerated transport, has been shown to reduce the development of internal breakdown in imported South African Santa Rosa, Gaviotta, Wickson and Kelsey plums, when compared with fruit ripened at prevailing warehouse temperatures (10). Australian plums were reported to ripen normally when the duration of exposure to a temperature of 32° F. was limited to a safe period, and was then raised to what was described as a minimum ripening temperature of 40° to 46° F. (11). This resulted in an extension of storage life over and above that which could be achieved by storing for a longer period at 32° F., followed by after-ripening at 65° F.

The publication of a paper by van der Plank and Rees Davies (12) on temperature and cold-injury to fruit drew attention to the fact that after relatively short periods in cold store, breakdown of plums, woolliness of peaches and pitting of Marsh grapefruit usually occur to a maximum extent at intermediate temperatures. The authors presented interesting cold-injury curves and put forward the suggestion that two factors were involved : an "equilibrium" factor, whereby lowering of temperature increases the disposition towards injury, and a "kinetic" factor, whereby the higher the temperature the sooner the injury becomes manifest.

Smith (3, 4) found that curves for internal breakdown in Monarch and Victoria plums could be obtained similar to those published by van der Plank and Rees Davies for Marsh grapefruit. He found it necessary, however, to differentiate strictly between two distinct types of disorder, the one occurring during and after storage at an intermediate range of temperatures (34°-45° F.), the other at a lower range (31°-37° F.). The former was termed " jellying ", and its symptoms were clearly the result of a process of abnormal ripening with a modification of the physical structure of the flesh, accompanied, as Hanes and Morris (12) have shown, by an alteration of the kind and amount of soluble pectin produced during senescence. The latter form of disorder, occurring at the lower range of temperature, was termed " internal browning " ; the symptoms appeared to result from a lethal injury to certain cells of the flesh during exposure to low temperature.

Experiments were made to clarify the distinct natures of these two types of disorder, inhibiting the one under conditions of temperature normally favourable to its occurrence by a treatment which promoted the occurrence of the other. It was during the course of these experiments that an interesting observation was made (4). If Victoria plums were stored at 31° F.—at which temperature jellying did not occur but internal browning was extensive—and were moved on the 17th day of storage to 65° F. for a period of 4 days, they could then be stored for a further 14 days at 31° F. without internal browning and with only a slight occurrence of jellying during subsequent ripening at 65° F.

The following account is a brief description of experiments carried out during the seasons 1945 and 1946 to confirm and to extend this finding.

I. SEASON 1945.

Victoria plums were picked on August 14th at an almost ripe, but still firm, stage and stored at once at 31° F. Comparable samples were transferred for 2 days, and others for 3 days, to a room at a temperature of 65° F., this treatment starting after the following numbers of days from the picking date : 0, 5, 10, 15, 20, 25, 30 and 35. The total period of storage was 37 days for the 2-day treatment and 38 days for the 3-day treatment, 35 days being occupied by storage at 31° F. At the expiration of the storage period the plums were cut transversely to the plane of the suture and the severity and frequency of occurrence of internal browning was assessed. An examination of duplicate samples was made on the 40th day, that is, after a further period of storage at 65° F.

Table I presents the data for the percentage of plums with internal browning for the 2- and 3-day treatments, both immediately on removal from store and (after post-storage ripening at 65° F.) on the 40th day from picking.

It will be seen from this Table that the interim high temperature treatment at the 15th day virtually eliminated internal browning when compared with the same treatment at the 35th day, which constituted a control. The 2-day and 3-day periods of treatment were equally effective. In the plums successfully treated there was no further development of internal browning during the post-storage period at 65° F. Intervention of the treatment on the 10th and 20th days both achieved almost completely effective control of this trouble : on days 0, 5, 25 and 30, control was only partially effective.

No jellying occurred in any of the samples. Another disorder, distinct from

TABLE I.

Internal Browning in Victoria plums stored at 31° F. in relation to interim exposure to high temperature treatment (65° F.).

	Percentage of plums with Internal Browning.							
	To 65° F. on day							
	0	5	10	15	20	25	30	35
Treated 2 days at 65° F.								
Examined on removal, 37th day ..	36	21	0	1	3	21	29	55
On 40th day	44	39	4	0	0	22	50	67
Treated 3 days at 65° F.								
Examined on removal, 38th day ..	15	11	1	1	3	11	26	55
On 40th day	47	31	6	1	0	15	25	68

internal browning and jellying, was noted, namely a condition of the flesh, which rendered it translucent instead of having the normal opacity of the tree-ripened fruit. The translucent flesh was juicy in contrast to the flesh of jellied plums which becomes dry and mealy. It is doubtful whether this condition, affecting only certain fruits—and these not completely—is to be considered a significant divergence from the normal course of ripening.

II. SEASON 1946.

Victoria plums were picked on August 24th, 1946, from another orchard, and at a slightly less ripe stage. They were graded into two groups :

(*a*) *More mature.* Coloured more than half-red, but including no fruits soft to the touch.

(*b*) *Less mature.* Coloured less than half-red, but excluding very small and blemished fruits.

The fruit was stored at once in trays at 31° F. The following treatments were given to comparable samples of about 50 plums :

Period of storage at 31° F. days	*Duration of interim treatment at 65° F.* days	*Treatment started after* days
40	1	0, 5, 15, 25, 40
40	2	ditto
51	1	ditto
50	2	ditto

There was thus a shorter and a longer period of storage at 31° F., and there was also treatment for a shorter and a longer period at the same five intervals from the date of picking. At the end of the storage periods, each fruit of the samples examined was cut in two transversely to the plane of the suture and the amount of internal browning assessed. The results of the examination are presented in Table II in which the percentage of fruits with internal browning is shown for each sample.

TABLE II.

Internal Browning in Victoria plums in relation to time and duration of an interim high temperature treatment, degree of maturity at picking, duration of storage at 31° F., and duration of post-storage ripening at 65° F.

Number of days at 31° F.	Degree of Maturity.*	Duration of treatment at 65° F.	Number of days post-storage at 65° F.	Percentage of plums with Internal Browning.				
				Treatment starting at day :				
				0	5	15	25	40
40	A	1	0	70	32	0	43	95
,,	,,	2	0	83	48	4	59	92
,,	,,	1	4	93	66	8	78	98
,,	,,	2	3	89	49	0	53	94
40	B	1	0	92	48	4	50	89
,,	,,	2	0	80	45	2	53	98
,,	,,	1	5	—	52	0	72	—
,,	,,	2	4	—	76	2	58	—
51	A	1	0	—	69	22	75	—
50	,,	2	0	—	95	16	56	—
51	,,	1	2	—	—	25	—	—
50	,,	2	2	—	—	38	—	—
51	B	1	0	—	—	47	—	—
50	,,	2	0	—	—	40	—	—
51	,,	1	3	—	—	68	—	—
50	,,	2	3	—	—	75	—	—

* A : More mature ; B : Less mature.

The results of the examinations may be summarized as follows :—

(1) *The effect of day of starting the treatment at high temperature.*

Treated at day 40 (Control). All samples contained a number of plums with more or less severe internal browning which approached 100 per cent. of the sample.

Treated at day 0. There was a slight reduction in the amount of internal browning as compared with the control samples.

Treated at day 5. There was a reduction by nearly one half in the amount of internal browning as compared with the control samples.

Treated at day 25. There was a reduction in the amount of internal browning comparable to that in the sample treated at day 5.

Treated at day 15. *Internal browning was almost completely eliminated* in the samples stored for 40 days.

(2) *The effect of duration of low temperature storage.*

In the second batch of fruit, removed after 50-51 days at 31° F., the amount of internal browning in the 15-day treatment was greatly reduced as compared with

that in the samples from all other treatments ; but at this stage internal browning was not entirely eliminated, in contrast to the result achieved during 40 days at 31° F.

(3) *The effect of the stage of maturity at picking.*

It will be seen from Table II that in the samples examined after 40 days' storage at 31° F., there were no significant differences in the percentage of plums with internal browning attributable to differences in the stage of maturity at picking. In those stored for 50-51 days, however, there was a higher percentage with internal browning in the less mature than in the more mature plums. The degree of internal browning was also more severe in the less mature fruit.

(4) *The effect of the duration of exposure to high temperature.*

There were no consistent differences to be observed in the percentage of plums with internal browning as between samples exposed for 1 day and those exposed for 2 days at 65° F. The plums exposed for 2 days, however, were of better quality than those exposed for 1 day ; they were more juicy and the flesh had a better appearance.

(5) *The effect of post-storage at 65° F.*

In general, there was not much increase in the percentage of plums with internal browning in the samples stored for 40 days at 31° F. during the after-ripening period at 65° F. for 2-4 days. There was, however, a tendency for a small number to become over-ripe. After storage for 50-51 days at 31° F., the subsequent period of after-ripening at 65° F. produced an increase both in number of plums affected and in the severity of internal browning.

(6) *Quality.*

The fruits treated successfully in these experiments had finally a good ripe red and orange coloration, characteristic of the variety ; they were juicy and of reasonably good flavour for stored plums. On cooking, they gave a palatable product with no abnormal taste. The samples of fruits that were more mature at picking time and had been subjected to 2 days treatment at 65° F. were preferable to the other samples. Even after 50-51 days of storage at 31° F., the plums which, as a result of the treatment, had escaped low temperature injury were palatable, especially after cooking. Very little translucence of the flesh was noted in successfully treated fruit, and after-ripening was mainly normal with only a slight tendency to jellying in a few individual plums. Wastage through rotting was negligible.

Discussion.

It has for some time been recognized that raising the temperature of storage has some effect in preventing the onset of internal breakdown in stored plums, which may possibly be attributable to a partial restoration of metabolic balance. It has not hitherto been shown, however, that a clear and incisive effect of such treatment is achieved at a definite and critical point in the senescent ontogeny of the fruit at a low temperature.

The results of the experiments described showed that internal browning of the flesh of the Victoria plum could be avoided if the fruit was exposed to a

temperature of 65° F. for a period of two days during a critical period at 31° F., namely, that between the 15th and 20th days from the start of storage. Exposure to 65° F. before or after the conclusion of this period failed to inhibit internal browning, though the degree of failure, as measured by the percentage of plums affected, varied with the point of time at which the treatment was applied. After the fruit had been exposed within the 15-20 day period, it remained free from internal browning for a further 15 to 20 days at 31° F., when internal browning again began to appear. It was not within the scope of these experiments, however, to ascertain whether further treatment, say in the 30-35 day period would once more inhibit the onset of internal browning ; but that may well be so.

In terms of storage life the effect of the treatment is further to prolong the life, by 50-75 per cent. over that previously obtained by storage at 34° F. There is also considerable improvement in quality. Nevertheless, certain difficulties must be overcome before such a method of treatment can be applied to plums stored in bulk, and it is therefore proposed to continue this work.

SUMMARY.

It is shown that complete control of internal browning of Victoria plums stored at 31° F. immediately after picking was achieved by the treatment of storing at 65° F. for 2 days, interposed at a point in time between the 15th and 20th day from the start. The plums so treated subsequently ripened normally after they had been stored for a further 15-20 days at 31° F. After more extended storage at 31° F. (50-51 days) internal browning was appreciable in amount, but still less than in the control samples treated at the 40th day. A period of treatment of 2 days rather than of 1 day was found preferable.

Plums of a range of maturity at picking (but excluding soft fruits) were found to have reacted favourably to the treatment when examined after 40 days at 31° F. The less mature plums, however, were of poorer quality than those of the more mature grades. After 50-51 days at 31° F., by which time the inhibition of internal browning was only partially successful, the less mature had suffered more extensive damage than the more mature fruit.

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VERTICILLIUM WILT OF THE HOP *(HUMULUS LUPULUS)*

II. THE SELECTION OF WILT RESISTANT VARIETIES

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EARLIER work by the writer (Keyworth, 1942) showed that Verticillium Wilt of the hop was spread by the dissemination of infected plant débris, and that field hygiene might therefore be expected to reduce the rate of spread of the disease. Strict field sanitation, however, including the use of soil sterilants, proved impracticable on a commercial scale ; and in spite of such precautions as were taken, the spread of disease continued both within affected farms and also to adjacent ones. Concurrent with the study of direct control measures, therefore, a search was started for varieties of hop that might be resistant to the disease.

As already reported (loc. cit.), a trial was first made of the eleven common commercial varieties by planting sets in a randomized experiment on a plot of naturally infected ground. This trial showed that none of these varieties possessed any marked resistance to the disease. There then remained for test the large range of new varieties bred by Professor E. S. Salmon, of Wye College, and also a few other new varieties bred on a commercial farm.

After initial experiments had shown that some of these new varieties might be resistant to Wilt, trials were started to test as many as possible of them each year. Any varieties showing high resistance in these primary selection trials were then to be tested further in small scale trials under commercial conditions ; and in subsequent years semi-commercial plantings would be made of those varieties which appeared both Wilt resistant and commercially acceptable.

The results obtained up to the present from the first two stages of these trials are now described.

PRIMARY SELECTION TRIALS.

The Wye varieties, bred by Salmon to produce types of improved commercial value from cultural and brewing points of view, were raised by crossing male and female hops from England, the U.S.A., Canada, Germany, etc. The progeny from these crosses were first grown at Wye, and the most promising plants were propagated vegetatively for transfer to East Malling, where they have been tested for cultural and brewing qualities in plots of fifteen or sixteen plants each. About 200 such varieties have been available at East Malling to provide young plants in sufficient numbers for randomized trials of Wilt resistance.

About thirty varieties have been tested each year in the primary selection trials. Previous work had shown that cuttings were unsatisfactory for such trials, owing to their relatively high mortality from causes other than Wilt. Bedded sets (i.e. one year old plants) were therefore used throughout, except in two experiments with

cuttings which were designed to eliminate the most susceptible varieties, but not to assess degree of resistance.

The primary selection trials were not made in disease areas in commercial gardens, owing to the uneven distribution of the pathogen. Plots that had been inoculated by the addition of diseased hop bine were therefore used. The bine was collected in the previous season, chopped into two-inch lengths and stored dry during the winter.

1943 Experiment.

This may be regarded as representative of the series of annual trials. The hop bine inoculum was scattered in March at the rate of one bushel per twenty yards of row, along trenches eight inches deep, nine inches wide, and spaced four feet apart (Fig. 1, Plate I). The trenches were then filled with soil and the test varieties planted in them shortly afterwards.

There were thirty-six plants of each of the thirty-one test varieties arranged in six randomized blocks. Clonal "indicator" plants of the susceptible Fuggle variety were spaced regularly throughout the experiment at the rate of one Fuggle to two test plants. These spaced indicators were omitted in other seasons, and control Fuggle plots were included instead. The bines from each plant were trained up a single 8 ft. string.

Of the 1,674 plants in the trial only eight failed to make some initial growth. Most of the others grew vigorously for some eight weeks, after which the first Wilt symptoms were seen on the lower leaves of some of the Fuggle indicator plants. Thereafter symptom records were made at approximately three-week intervals until the end of September when a final assessment of the condition of the plants was made. The evaluation of the resistance of a variety was made largely on this final assessment, but the intermediate records were used to determine whether any plants showed partial recovery and as a check for accidental damage. After the final symptom records had been made, all the bines were cut two inches above soil level and examined for brown discoloration of the wood.

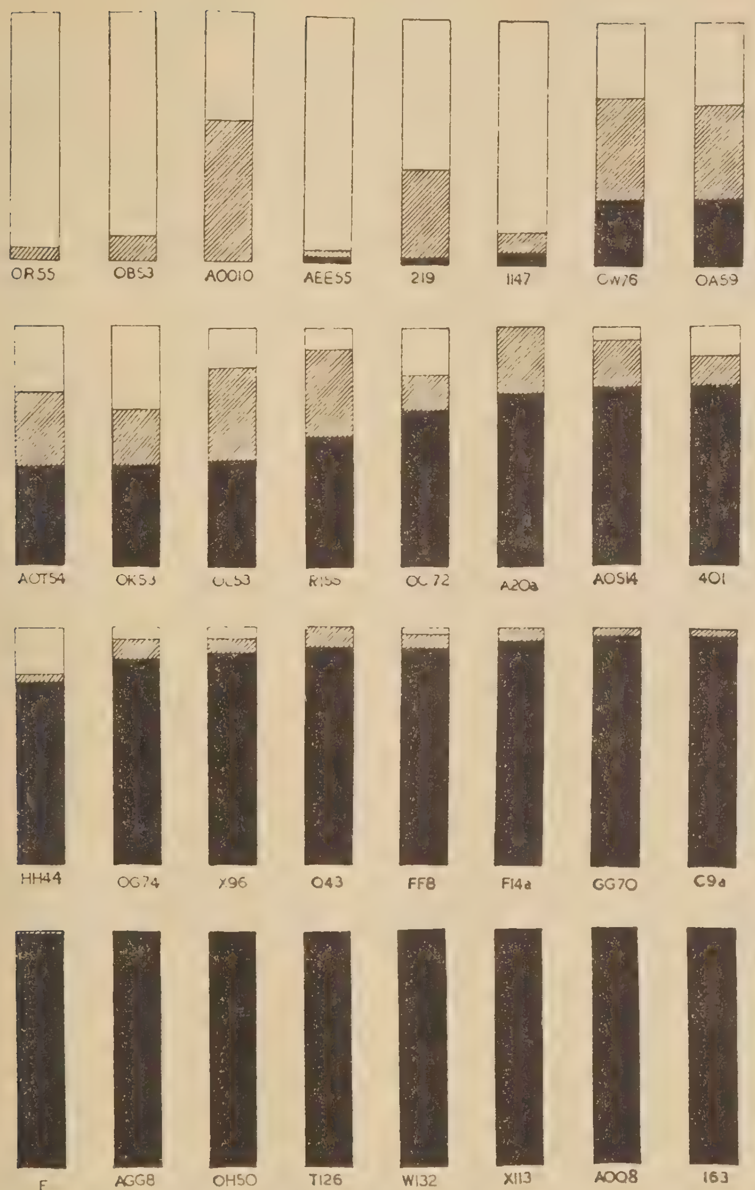
The final results are expressed in diagrammatic form in Text Fig. 1. As will be seen, the tested varieties form a fairly evenly graded series from those showing no external symptoms to those in which every plant was wilted ; but no variety was completely immune.

The apparently healthy bines of the varieties AEE55, AOO10 and OR55 were further tested by the incubation of portions of them in the laboratory. Verticillium condiophores developed on the bines from eighteen of the eighty-two plants so tested, thus indicating that some of these apparently healthy plants were infected.

The high resistance of some of the varieties was clearly demonstrated by comparison with the adjacent Fuggle plants. Figs. 2 (Plate I) and 3 (Plate II), showing a general and a close-up view of a plot of the variety AEE55 and the interplanted Fuggle, illustrate this feature.

1944-46 Experiments.

The plots of infected soil used for the 1943 trial were used again in subsequent years. The rows were evenly reinfected each season in order to eliminate any possible differences in infectivity caused by the growing in them during the previous year of varieties of differing resistance. The complete results of the 1943-46 primary selection trials are shown in Table I.



TEXT FIG. 1.

Diagram of records from the 1943 Primary Selection Trial.
Each column represents one variety.

Black portion :	No. of wilted plants.
Shaded portion :	No. of plants containing brown wood.
White portion :	No. of apparently healthy plants.

TABLE I.

Primary selection trials of new varieties, 1943-46.

% Wilt.	1943.	1944.	1945.	1946.
0–10	AEE55. AOO10. OB53*. OR55. 219*. 1147*.	—	AOM47. EE29. OM103. X111.	(AOM47). (OR55).
11–20	—	—	FF21. ADD50.	OM26. OW3. (1147).
21–30	OA59. OW76.	OJ47.	AAA3. AOR10. H36. OI47. OL60. OT156.	—
31–40	AOT54. OK53.	R1/30. R2/1.	OA90. OG13. OR141. OW33. R1/50a.	OM32.
41–50	OL53. R1/55.	AII16. AOY18. B16.	AOQ12.	—
51–60	OC72.	AOR35. HH102. R3/97a.	EE64.	—
61–70	HH44. 401.	AOX19. OH1.	OH104. P19. WFF12.	H56. R1/37.
71–80	A20a. AOS14. FF8.	CC77. Z3. 347.	—	AOO25. AOP3. EE36. 287a.
81–90	C9a. GG70. OG74. Q43. X96.	DD97. R4/7a. X69.	OV59. R2/25a. R4/101a.	—
91–100	AGG8. AOQ8. F14a. OH50. T126. W132. X113. 163.	AA81. AA101. ACC5. AOS6. AOS9. BB28. EE69. EE77. FF60. GG60. K1. OB41. OD18. OF81. OH72. OH77. OR88. OS98. R1/38a. R2/23a. R3/100. V32. WFA111. Y103.	AEE44. DD12. OL12.	ADD4. AOO36. HH50. II149. OB9. OH7. OL26. OL63. OK40. OR38. WFA12. WFA90. WFD20. Z63. 109a.

NOTES. 1. The variety 1147 was raised on a commercial farm ; the code numbers of the other (Wye) varieties are related to their position in the Wye nursery and do not indicate parental affinities.

2. The varieties indicated by an asterisk in the 1943 trial were selections from a previous small scale trial, and those in brackets in the 1946 trial were previously tested varieties, included as indicators.

In addition to the varieties listed in Table I, the following were rejected after non-randomized tests :—
AEE36, AFF27, AOO1, AOO13, AOP2, AOP6, AOP24, AOQ29, AOQ39, AOR36, AOR45, AOS31, AOT36, AOW31, DD5, DD100, G15a, GG64, K24, K31, K55, L11, L21, M35, N15, OB108, OC16, OD100, OE86, OF27, OG3, OH44, OL33, OM17, OM50, OO21, OO63, OO71, OP3, OP21, OQ4, OQ22, OR76, OS76, OS141, OT22, OT91, OT100, OV80, OW28, OW92, OW94, OX88, OZ79, R1/9, R1/94a, R2/5, R4/40a, R4/80a, RW10, V94, W506*, W932*, W978*, W1200*, WFB26, WFF28, WFF108, WFG19, Z62, 9a, 170a, 234a, 309, 334a, 342, 384a.

Thus, during the years 1943-6, a total of 201 new varieties has been tested.

SMALL SCALE COMMERCIAL TRIALS.

The varieties showing least Wilt in each annual selection trial were further tested in succeeding years by field trials in disease areas on commercial farms. Shortage of planting material limited the size of the experiments and it was decided that the stock could be used to the greatest advantage by conducting small trials of an observational nature on a number of farms rather than by concentration of the material in a large trial on one farm. All the trials were randomized, and susceptible Fuggle controls were included in order to minimize irregularities due to the effects of uneven soil infection and to provide some basis for a comparison of the different sites. Bedded sets were used throughout and they were planted in the spaces formerly occupied by mature plants after these had been carefully grubbed and the soil replaced. In some cases a few healthy plants were grubbed in order to arrange the experiment in a convenient shape, but in every case the area used had contained a high proportion of wilted plants. In four experiments the test varieties replaced scattered wilted plants.

1943 TRIAL.

Farm A (*Site 1*). One experiment was laid down in 1943 on a site which had contained about 75 per cent. of wilted plants in 1942. In this trial the varieties OB53 and 1147, which had been selected from a small preliminary trial in 1942, were compared with the varieties Fuggle and Tutsham, the latter being included as it had shown some indications of slight resistance in established commercial gardens. The results for the three years 1943 to 1945 are given in Table II.

TABLE II.

Small scale commercial trial. Farm A (*Site 1*).

Variety.	No. of plants.	No. wilted.†		
		1943.	1944.	1945.
OB53	39	0	2	0
1147	37	4	4	7
Tutsham	38	25	28	31
Fuggle	38	27	30	37

* Varieties bred on a commercial farm.

† Records were made afresh in each season of all the plants in this and other trials. The number recorded as wilted includes those which had died in a previous year. No replanting was carried out.

These results show that most of the plants of the varieties OB53 and 1147 remained free from external symptoms for three years in ground which was sufficiently heavily infected to induce wilt in almost all the Fuggles. No marked resistance was shown by the Tutsham variety under these conditions. On examination in September, 1943, eleven plants of the variety OB53 were found to have brown wood in their bines, and isolations of *Verticillium albo-atrum* were made from them. As none of them showed severe Wilt symptoms in subsequent years it is apparent that plants of this variety can remain free from external symptoms even though containing the fungus. All the other resistant varieties have been found to behave similarly. It will also be noted that two OB53 plants showing Wilt symptoms in 1944 were free from such symptoms in 1945.

1944 Trials.

Farm B. This trial was laid down in a Fuggle garden in which there had been an extremely severe outbreak of Wilt and all the plants in the experimental area had shown severe Wilt in 1943. All the test plants were two years old and, with the exception of the Fuggles, had already been grown for one year in heavily infected ground from which they had been dug for planting in this trial. Records for two years are given in Table III.

Table III.

Small scale commercial trial. Farm B.

Variety.	No. of plants.	No. wilted.	
		1944.	1945.
OB53	60	1	1
AEE55	30	4	1
OR55	30	18	2
219	30	17	9
1147	30	21	15
AOO10	30	24	21
Fuggle	60	60	60

The results indicate that the varieties OB53 and AEE55 were highly resistant in an area in which all the Fuggle plants were severely wilted. They also show that all the varieties except OB53 and Fuggle contained fewer wilted plants in 1945 than in 1944 ; this is particularly striking with OR55 and may possibly be a seasonal effect. In this connection it is noteworthy that the primary selection trial in 1944 contained no varieties showing less than 20 per cent. of wilted plants. The effect might also, of course, be related to the fact that the test plants had already been grown during 1943 in infected soil.

Farms C, D and E. Three small trials were laid down on these farms using the varieties AEE55, OR55, AOO10 and Fuggle. The sets were planted on the sites of scattered wilted plants which had been grubbed in 1943. The 1944 and 1945 records are given in Table IV. They demonstrate the complete absence of wilt

Table IV.

Small scale commercial trial. Farms C, D, E.

Variety.	Farm.	No. of plants.		No. wilted.			
				1944.		1945.	
AEE55	C	10	30	0	0	0	0
	D	10		0		0	
	E	10		0		0	
OR55	C	10	30	3	7	5	11
	D	10		2		2	
	E	10		2		4	
AOO10	C	10	30	3	12	3	17
	D	10		3		8	
	E	10		6		6	
Fuggle	C	18	54	13	42	16	52
	D	18		14		18	
	E	18		15		18	

in the variety AEE55 on all the farms. Neither OR55 nor AOO10 showed high resistance although both were less severely affected than the Fuggle controls.

Farm F. The four varieties OB53, 219, OW76 and Fuggle used in this trial were planted in the spaces formerly occupied by scattered wilted plants. The results from the 1944-45 records are given in Table V.

Table V.

Small scale commercial trial. Farm F.

Variety.	No. of plants.	No. wilted.	
		1944.	1945.
OB53	25	0	0
219	25	3	6
OW76	25	7	12
Fuggle	25	18	22

1945 Trial.

Farm A (*Site 2*). The site of this trial had contained about 60 per cent. of wilted plants in 1943 and it was then grubbed and cropped with runner beans for one year. The varieties used were OB53, OM26 (a bud sport of OB53), 219, 1147 and Fuggle, 100 sets of each being planted in five randomized blocks. A few sets died from causes other than Wilt, thus slightly reducing the effective number of plants.

The results during 1945 and 1946 are shown in Table VI. They again demonstrate the high resistance of the variety OB53, and also of the bud sport OM26. The varieties 219 and 1147 also showed marked resistance to the disease.

TABLE VI.

Small scale commercial trial. Farm A (Site 2).

Variety.	No. of plants.	No. wilted.	
		1945.	1946.
OB53	97	0	3
OM26	98	0	2
219	100	0	5
1147	94	0	12
Fuggle	95	19	66

Crop records were taken from this experiment in 1946. The dry weights of the hops are shown in Table VII together with their equivalents per acre of 1,200 hills (calculated on 100 hills per variety). The records demonstrate that the Fuggle plots gave a quite unecomonic crop whereas all the other varieties gave one which compared favourably with that which would have been produced on uninfected ground by similar two-year-old plants.

TABLE VII.

Crop records. Farm A (Site 2).

Variety.	Crop weight (lb.).	Equivalent per acre (cwt.).
OB53	96	10¼
OM26	106	11¼
219	151	16¼
1147	123	13¼
Fuggle	38	4

SUMMARY OF SMALL SCALE COMMERCIAL TRIALS, 1943-1946.

The accumulated records on the main varieties tested are summarized in Table VIII. The totals of wilted plants are those noted on the last occasion on which records of the trials were made.

TABLE VIII.

Summary of results from small scale commercial trials.

Variety.	No. of plants.	No. wilted.	%
OB53	221	4	2
OM26	98	2	2
AEE55	60	1	2
219	155	20	13
1147	161	34	21
OR55	60	13	22
AOO10	60	38	63
Fuggle	272	231	85

In view of the considerable variation between the trials and of the small numbers of plants involved, these results cannot be regarded as indicating accurately the grades of resistance. However, they show conclusively that under the conditions of the experiments the three varieties OB53, OM26 and AEE55 were highly resistant to Wilt, and suggest that the varieties 219, 1147 and OR55 may be regarded as moderately resistant.* AOO10 seems to be of low resistance only.

DISCUSSION.

The results of the small scale commercial trials do not agree closely with those of the 1943 preliminary trial from which the test varieties were selected. Whereas all the selected varieties showed somewhat similar degrees of wilting in the preliminary trial (see Text Fig. 1) the subsequent trials revealed considerable differences between them. It thus appears that although this preliminary trial enabled promising varieties to be selected it did not indicate the precise extent of disease likely to occur when they are planted in Wilt-infected areas in commercial gardens. Trials of the selected varieties under the latter conditions are thus necessary before their resistance can finally be assessed.

As all the resistance trials have been set out with young plants put into already heavily infected soil they have assessed the value of the test varieties for replanting infected areas, but they have given no direct information on the resistance to *introduced* infection of well-established plants growing in uninfected soil. As already reported, the variety Tutsham is regarded by some growers as showing slight resistance under these conditions, it having been observed that while adjoining Fuggle gardens were severely affected, the Tutshams were less attacked. Observations on the variety 1147 have also given some information on the matter. This variety was raised on a commercial farm near Paddock Wood on which a severe outbreak of Wilt subsequently occurred. A block of the variety had been established in one garden (together with other varieties on each side) for about fifteen years when the garden became infected with the disease. Within seven years the other varieties had become severely affected by Wilt, but the plants of the variety 1147 remained completely free from external wilt symptoms. When sets of this variety were planted in heavily infected soil, however, 21 per cent. showed symptoms, indicating only moderate resistance under these conditions. It seems probable, therefore, that tests of varieties by planting sets in infected soil may give too low an estimate of their resistance once they have become fully established. The effectiveness of resistant varieties in withstanding the disease may therefore be increased if they are planted in infected ground after a fallowing period, or planted in uninfected ground on affected farms.

It may be concluded that the use of resistant varieties provides some prospect of a control of Wilt. The results from the commercial trials, however, suggest that the varieties selected up to the present may vary in their reactions to the disease on different farms, and it is thus possible that the effect of soil conditions or the origination of new strains of *Verticillium albo-atrum* may cause a breakdown of resistance.

As described above, none of the varieties has proved to be immune, and plants

* Notes on the characters of all the selected varieties are being published in the Annual Report of the East Malling Research Station for 1946 (published 1947).

of all of them may tolerate the presence of the pathogen in their bines. It is not yet known whether an infected cutting of a resistant variety can transfer the pathogen to the soil in which the cutting is planted. If this is so, however, the planting of such infected cuttings may either infect clean ground or possibly introduce a different strain of the Verticillium into already infected soil. In order to avoid this danger it is advisable that all hills from which planting stock is to be taken for general distribution should be on uninfected ground.

ACKNOWLEDGMENTS.

The writer gratefully acknowledges the help and encouragement given throughout this investigation by Professor E. S. Salmon and Dr. R. V. Harris, and also the collaboration of Mr. F. H. Beard, who was responsible for the propagation of the very large number of plants used. Thanks are also due to many other colleagues, notably to Mr. S. C. Pearce for his advice on the layout of the experiments and to Miss M. Bennett and Miss M. M. Hitchcock, who undertook much of the planting and recording work. Finally, the writer must record his debt to the many growers who have collaborated in this work and particularly to Messrs. W. Whitbread & Co., Ltd., on whose farm many of the trials were carried out. He is specially indebted to the staff of this firm who spared no effort to make the trials a success.

SUMMARY.

A description is given of the methods by which hop varieties have been selected for resistance to Verticillium Wilt and further tested in commercial gardens. The tests have shown that some of the Wye varieties and one variety bred on a commercial farm possess marked resistance to the disease.

REFERENCE

Keyworth, W. G. (1942). Verticillium Wilt of the hop (*Humulus lupulus*). Ann. Appl. Biol. **29**, 346-357.

(*Received* 14/2/47.)

PLATE I.

Fig. 1
Soil inoculation method of testing new varieties for resistance.

Fig. 2.
Plot of variety AEE55 and accompanying Fuggle in 1943 resistance trial.

[*face p.*108

PLATE II.

FIG. 3.

Close-up view of part of plot shown in Fig. 2 to illustrate the contrast between the one dead Fuggle (F) plant and the three apparently normal AEE55 plants.

AN EXTENDED TRIAL OF SEEDLING CHERRIES

By M. B. CRANE
John Innes Horticultural Institution

SINCE the year 1921 breeding work with cherries has been carried out at the John Innes Horticultural Institution for the purpose of genetical study. The seedlings raised have been grown on their own roots, and the majority reached maturity, indeed cropped heavily, in their sixth year. The fruits of several of the seedlings were attractive in size and flavour, and it was decided to propagate and test these for commercial possibilities. It was not possible to carry out such a test at Merton owing to lack of space ; and, apart from this, experience had shown that it would have been a doubtful proposition because, with a relatively small area of cherries, birds take a heavy toll of the fruit long before it reaches maturity. For the latter reason it was also concluded that it would not be satisfactory to attempt to have the seedlings included in the National Fruit Trials at Wisley. The Authorities of the Kent Farm Institute were therefore communicated with, and their reply was prompt and helpful. Not only were they prepared to provide for a trial of the seedlings and carry out the cultivations, but they also arranged for Mr. Hart, now Principal of the Institute, to come and see the seedlings, and he took part in their selection.

The following were the seedlings that were selected and propagated :

Number.	*Parentage.*
42	Bigarreau de Schrecken × Governor Wood.
185	Knight's Early Black × Bigarreau Napoleon.
193	Knight's Early Black × Bigarreau Napoleon.
404	Bigarreau de Schrecken × Elton.
413	Emperor Francis × Bedford Prolific.
414	,, ,, × ,, ,,
418	,, ,, × ,, ,,
419	,, ,, × ,, ,,
427	,, ,, × ,, ,,
490	Elton × Bigarreau de Schrecken.
516	Bigarreau Napoleon × Bigarreau de Schrecken.
519	,, ,, × ,, ,,
573	Late Black Bigarreau × Governor Wood.
659	Belle Agathe × Bigarreau Napoleon.

The fruits of these seedlings ranged in season of ripening from early to very late, and they varied in colour, texture of flesh and in other respects.

The East Malling Research Station supplied rootstocks of their two clones F2/1 and F12/1, and the seedlings were worked (an equal number of each) on each rootstock by budding approximately at ground level. At the same time trees of the varieties Amber Bigarreau, Bigarreau Napoleon, Early Rivers, Governor Wood, Roundell and Waterloo were raised. These were worked high on the same rootstocks, F2/1 and F12/1 ; the stems of these varieties, therefore, were formed by the root-stocks, whilst those of the seedlings were their own.

A randomized trial of the seedlings and the varieties was designed by Mr. T. N. Hoblyn, of East Malling, and the trees were planted during the 1933-34 planting season.

During the early years of the trial the plantation was inspected by a small committee each year, and the growth and health of the seedlings and the varieties were recorded. Later, when the trees came into bearing, they were inspected two or three times during the fruiting season. Very early in the trial it was evident that there were pronounced differences between them in habit of growth and other characters, as well as in susceptibility to attack by Bacterial Canker (*Pseudomonus mors-prunorum*). This disease occurred both among the varieties and the seedlings. In some of the latter the attack was severe and mortality high; in others it was relatively slight and mortality was low. Infection with this disease appears to occur most readily through wounds, and it is commonly stated that high-worked trees are preferable to low-worked trees because the stock is usually more resistant than the scion. When the stem becomes infected the canker often spreads, completely girdling it and killing the tree. As previously stated, the seedlings were low-worked, which, in effect, means that they were more liable to infection and attack than the high-worked varieties. Hence the seedlings experienced a more severe test than the varieties, and it is probable that those which became attacked only slightly are appreciably resistant to the disease.

Most of the seedlings used were raised in 1921, propagated in 1931 and planted in the trial in 1933. Thus it is now 25 years since they were raised, and 15 years since they were being prepared for trial.

A few years after the trials were planted the Committee of the National Fruit Trials—which are conducted jointly by the Royal Horticultural Society and the Ministry of Agriculture—formed a sub-station for cherries at the Kent Farm Institute, and these trials then became included officially in the National Trials.

In 1945 it was decided that two, and in 1946 that three other seedlings were worthy of commercial introduction, and these five have been given names. In the latter year, in addition to being seen by the National Fruit Trials Committee, the trials were inspected and reported upon by a sub-committee of the Fruit and Vegetable Committee of the Royal Horticultural Society, and samples of fruits were also sent to the Committee in London. One of the seedlings, Merton Bigarreau (193), was awarded a First Class Certificate, and the other four, Merton Favourite (185) Merton Heart (404), Merton Premier (418), and Merton Bounty (490), received Awards of Merit.

It is proposed to distribute propagating material (wood for budding purposes) of these new seedling varieties as it becomes available. A limited amount will be available in 1947; in subsequent years it should be in more plentiful supply.

Pollination tests have shown that Merton Bigarreau is in Group II* and Merton Heart in Group VI. The pollination relationships of the other three have not yet been worked out, but since a seedling is always compatible with its female parent, and therefore with all others in the same group as its Mother, it follows that Merton Favourite is compatible with all varieties in Group I, Merton Premier with all in Group III and Merton Bounty with all in Group VI. Therefore, although our knowledge is incomplete, it is possible when planting these new varieties to arrange for effective cross-pollination.

* The names of the varieties in the Groups mentioned are given in John Innes Leaflet No. 4:—The Fertility Rules in Fruit Planting.

When considering the siting and layout of fruit crop trials the question is sometimes asked: Is it necessary for them to be carried out in an area where the crop is commercially grown? This may not be so with all crops, but with cherry trials the writer considers it essential to do so, if only to avoid or reduce the ravages of birds. Apart from this, it has been clear throughout the course of these trials that they have gained much by being situated in an area where cherries are extensively grown. They have been readily accessible to and visited by neighbouring expert growers whose advice and criticism has been of the greatest value, and whose long experience enables them to see quickly defects as well as good points. Furthermore, the seedlings can readily be compared with the best standard varieties that have been grown for a long time in the district.

The members of the Cherry Trials Committee were: Drs. R. G. Hatton and H. M. S. Montgomery, Messrs. M. B. Crane, S. R. Dixon, N. H. Grubb, R. Hart, T. Neame and J. M. S. Potter, Secretary of the National Fruit Trials Committee.

(*Received* 20/2/47.)

THE NEW MERTON CHERRIES

By ROBERT HART
Kent Farm Institute

In the previous article, Mr. Crane has given a brief outline of the trials that are being carried out at the Kent Farm Institute, the purpose of which is to compare, in respect of habit, tree performance, quality of fruit, etc., a selection of the seedling sweet cherries raised at Merton with some of the well-known varieties in general cultivation.

The selected seedlings were grouped in three sections according to the season of fruiting as early, mid-season and late. Six well-known varieties, a black and a white in each group, were chosen for purposes of comparison, viz. Rivers Early and Governor Wood (early), Waterloo and Amber Heart (mid-season), Roundel and Napoleon Bigarreau (late).

Thirteen seedlings were planted in the original trial, and of these, five have already been selected by the National Fruit Trials Committee for further trial. In the description of these five varieties given later in this article, there may appear to be a too abundant use of superlatives, but that is not so. When I visited the John Innes Horticultural Institution in 1931 to see Mr. Crane's seedlings, I soon came to the conclusion that, amongst them there were individuals that were very much superior in size and flavour of fruit to anything I knew of in commercial cultivation, and that if, when propagated on commercial stocks and grown under commercial conditions, they made good trees, carrying reasonable crops, they would form valuable additions to the list of varieties suitable for commercial cultivation. With the five varieties already selected for extended trial, this early promise has been amply fulfilled, whilst amongst the other seedlings still under observation, there may yet be others worthy of extended trial. The Cherry Trials Sub-Committee consists of an exacting body of men, and before recommending a new variety for extended trials, they have had to be convinced that it is not merely as good as, but is definitely an improvement on an existing well-known variety of the same season and colour in at least one of the characteristics that contribute to the make up of a good commercial variety of sweet cherry. The characteristics are : size and quality of fruit, size and regularity of crop, vigour, resistance to diseases such as Bacterial Canker, Brown Rot and Blossom Wilt, and so on.

The trees in the trial have not been pampered in any way, rather the reverse. They were just coming into bearing in the early days of the war and the trial, like all orchards of the same age, suffered the vicissitudes inseparable from wartime emergency, such as being under-cropped with roots or vegetables, although it was known that by all the canons of good cherry growing the orchard should have been grassed down ; and even when grassed down, it was grazed with sheep in order to produce a little more mutton, when one's better judgment said that the grass should have been kept under control by the use of a gang mower.

In the early 1930's, when this trial was being planned, Bacterial Canker (*Pseudomonas mors-prunorum*) was recognized as one of the most serious troubles of the plum and cherry grower, being annually responsible for the deaths of large numbers of young trees. There is a marked variation in the susceptibility of different varieties to the attacks of this organism, and at that time it was thought that the most

promising method of dealing with this disease was in the selection of varieties showing marked resistance to it. In view of this, no attempt at control by spraying was made in the early years of the trial, and infection was allowed to take place naturally. In fact, the seedlings had a very severe test, because the Merton trees were all worked at ground level, whereas those of the named varieties with which they were compared were worked at standard height. This tended to render the former more liable to complete girdling by the attacks of the parasite. Sources of infection were always present, because not only did one or two of the seedlings prove highly susceptible, but deaths also took place amongst the trees of the known varieties used as controls, varying from the relatively resistant Governor Wood, with 22 per cent. mortality, to the very susceptible Napoleon, with 81 per cent. Incidentally, this brings out the necessity of a continued search for new varieties that are an improvement on existing ones ; valuable as the five new varieties will prove, they are all black cherries, and there is still room for a late mid-season white cherry that will produce regular crops of large fruits like those of Napoleon, but with the quality and resistance to Bacterial Canker of Crane's Merton Heart.

A Table is given below showing the mortality in the trial caused by Bacterial Canker, but in studying this it must be borne in mind that, while resistance to this disease was one of the characteristics it was intended to study, this trial was not designed solely for that purpose. Consequently, whilst the figures can be taken as indicative of the natural resistance or otherwise of an individual variety *under the conditions prevailing in the trial*, they are reliable only within fairly wide limits. For instance, while it is quite safe to say that Merton Heart (Seedling 404) showed much greater resistance than Seedlings 516 or 519 (or, for that matter, than any other variety included in the trial), it is not safe to argue that, because in this trial Seedling 519 (with a death rate of 53 per cent.) showed an apparently greater resistance

Merton Seedling Cherry Trial.
Mortality figures 1934-43.

Varieties.	Trees.		Re-plants 1934-43.		Percentages of deaths.
	No. planted.	No. alive.	Planted.	Alive.	
Amber	14	8	5	1	53
B. Napoleon	6	2	1	—	81
Early Rivers	10	7	1	1	27
Governor Wood ..	8	6	1	1	22
Roundel	6	3	2	2	38
Waterloo	14	8	4	—	56
Merton Favourite (185) ..	8	4	1	1	44
Merton Bigarreau (193) ..	8	4	1	1	44
Merton Heart (404) ..	12	11	1	1	8
Merton Premier (418) ..	6	—	3	3	67
Merton Bounty (490) ..	8	6	—	—	25
413	9	—	3	3	75
414	7	4	2	2	33
419	8	—	1	1	89
427	8	1	—	—	88
516	5	—	4	3	67
519	5	—	3	3	63
573	7	5	—	—	29
659	9	5	—	—	44

than Seedling 516 (with 67 per cent.), it possesses, in fact, greater natural resistance.

Since the planting of the trial, a considerable amount of research work has been done at East Malling Research Station and elsewhere on the control of Bacterial Canker, and it is known that a large measure of control can be obtained by spraying with copper fungicides ; hence, natural resistance to the disease does not now assume quite so much importance as it did when the trial was being planned. At the same time, it is right to point out that resistance *is* still important. Given two varieties of about the same season and of approximately equal value in other respects, a commercial grower would naturally select the variety with the greater resistance ; for not only would his chances of the complete control of the disease in *that variety* be greater, but the introduction of a more susceptible variety would increase the risk of infection occurring in trees of other varieties present—a very important consideration with sweet cherries with which, in order to secure adequate pollination, there has to be a very intimate mixing of varieties.

Description of the five Merton seedlings.

The seedlings are arranged here approximately in order of ripening, but there is a certain amount of overlap between Merton Premier, Merton Favourite and Merton Bounty, added to which, as young trees only are available, it is not an easy matter to decide when the fruit should be picked ; hence a more prolonged study of the trees may lead to a revision of this order. Similarly, at a later date, it will be possible to give reliable information in regard to the time of flowering of all these varieties and also as to into which of Crane's intra-incompatible groups the varieties Favourite Premier and Bounty respectively fall.

Merton Heart. (Seedling 404.) Big. Schrecken × Elton Heart. Crane's intra-incompatible Group VI.

Season : Second early, about equal to Circassian.

Habit of tree : Vigorous and very upright.

Cropping : There is an absence of long laterals and the fruit is borne in clusters close to the main limbs which gives a deceptive appearance ; in reality this variety is a very heavy and consistent cropper.

Fruit : Colour, deep purplish crimson, becoming jet black when fully ripe. Flesh, dark red and juicy. Flavour, extremely rich. Size and shape, large, heart-shaped.

General : So far this variety has shown itself to be markedly resistant to attacks of Bacterial Canker and also to Blossom Wilt (*Sclerotinia laxa*). It shows its Elton parentage in the shape and very high quality of the fruit. On its inherent merits alone it is well worth further trial, but coming in season just after Rivers Early it fills an obvious gap and is consequently regarded as a very valuable addition to the list of cherries available to the commercial grower.

MERTON BOUNTY. (Seedling 490.) Elton × Big. Schrecken.

Season : Early mid-season.

Habit of tree : Vigorous, upright, spreading.

Cropping : Moderate to heavy and consistent.

Fruit : Colour, purple mottled with lighter freckles. Flesh, pale crimson and juicy. Flavour rich. Size and shape, large, heart-shaped.

General : As a black cherry, Bounty will come in after Circassian at a season when there are not many good blacks ; but probably, when it gets into commercial cultivation, it will seldom be allowed to get ripe. At about the time "green" Governor Wood fruit is being put on the market that of Bounty has an attractive pink cheek on a white ground and is quite edible. Although the quality and flavour is nothing like as good then as when fully ripe, it is still better than the general run of Governor Wood, which it also excels in size.

MERTON PREMIER. (Seedling 418.) Emperor Francis × Bedford Prolific.

Season : Early mid-season, about the same time as Noir de Guben.

Habit of tree : Moderate in vigour, upright, spreading.

Cropping : Moderate but consistent.

Fruit : Colour, deep crimson purple. Flesh, dark red and firm. Flavour, very good. Size, large; bigarreau shape.

General : Premier should prove a valuable addition to the list, coming in at a time when there are not many varieties combining good size and attractive appearance with regularity of crop.

MERTON FAVOURITE. (Seedling 185.) Knight's Early Black × Big. Schrecken.

Season : Mid-season, just before Waterloo.

Habit of tree : Moderate in vigour, much branched, somewhat spreading. Very like Waterloo.

Cropping : Moderate to heavy and consistent.

Fruit : Colour, deep purplish crimson darkening to jet black when fully ripe. Flesh, dark purplish crimson and very juicy. Flavour, excellent. Size, large ; shape oval.

General : Favourite may fairly be described as an improved Waterloo, which it closely resembles. It is a little earlier in season, much larger in size ; so far in the trials it has been consistently a more heavy cropper and it makes a larger tree. The fruit has all the lusciousness that makes Waterloo such a popular cherry.

MERTON BIGARREAU. (Seedling 193.) Knight's Early Black × Big. Napoleon Crane's intra-incompatible Group VI.

Season: Late mid-season, about the same time as Napoleon.

Habit of tree: Vigorous and very spreading.

Cropping: Regular and very heavy.

Fruit: Colour, deep mahogany. Flesh, firm and dark red. Flavour, extremely rich. Size, large; shape, bigarreau.

General: Merton Bigarreau is probably the most important of Crane's introductions. It has the size and quality of a Noble without the astringent under-flavour the latter often exhibits when not grown under absolutely tip-top conditions. Combined with this it appears to have the robustness of an Amber, or even more so, as the Merton Bigarreau trees are all larger than the trees of Amber in the trial. If it has a fault it is a tendency to over-crop and thus reduce the size of the fruit; it will probably respond profitably to very heavy manuring. The remark of Raymond Bush when he tasted it: " . . . the best and finest cherry seen for many a long day", can be applauded—but then Merton Heart and Merton Favourite were both past their best when he tasted Merton Bigarreau!

The accompanying photographic reproductions of the fruits and stones of these varieties (Plates I, II, III) are shown natural size.

(*Received* 20/2/47.)

PLATE I

FIG. 1. Merton Heart.

FIG. 2. Merton Premier.

[face p. 116

FIG. 3. Merton Bounty.

FIG. 4. Merton Bigarreau.

PLATE III.

FIG. 5.
Merton Favourite.

THE APPLICATION OF BIOLOGICAL OBSERVATIONS ON WILD AND NATURALIZED SPECIES AND VARIETIES OF FRUIT TREES TO THE STUDY OF FRUIT TREE ROOTSTOCKS

A PRELIMINARY STUDY OF SOME PRUNUS SPECIES

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INTRODUCTION.

One of the most important results established for fruit tree rootstocks at East Malling is that the nature of the rootstock can determine the degree of vigour of the scion. With apples the experiments have extended over a period of 30 years, and they have given a complete series of data from which classification of the existing rootstocks could be made in relation to the behaviour of scions worked on them. By breeding from the rootstocks, families of seedlings have been obtained which have shown a great range of variation as regards their action on scions worked on them (Tydeman, 1937, 1943). Among these seedlings there have been found a few individuals that exert an extreme dwarfing influence on the scion.

Recently it was found at East Malling (Beakbane and Thompson, 1939) that the dwarfing influence possessed by apple rootstocks was correlated with the amount of living tissue in the roots, the most dwarfing type of rootstock having the greatest proportion of living tissue in the root. Most of the living tissue in the apple root is present in the bark, and determination of the per cent. bark in the root was found to give (within a certain margin of error) a measure of the influence of the rootstock on the scion. This made possible for the first time prediction, without field trials, of the effect that a given rootstock would exercise on a scion.

The present work was undertaken jointly with the object of finding out to what extent rapid methods of prediction could be used either for the classification of existing rootstocks of unknown type or for a preliminary sorting of material destined to be tested by actual trial. The work described in what follows started with certain observations on the extreme dwarfing types of apple rootstock raised as seedlings at East Malling. It is considered desirable to bring to the notice of pomologists at this early stage in the work, the observations already made, because it is felt that chance observations on the occurrence of extreme types of individual plants may be of great value for experimental work and might lead finally to results of economic value.

During the last three years it has been observed in apples grown specially for the present study that in the extreme dwarfing types the habit of growth of the rootstock's own stem is similar to that of the scion worked on it ; this was found

to hold good for very vigorous rootstocks also. These extreme types provide a means of further investigation of the nature of the small, precociously bearing, fruit tree. In the apple rootstock, as has been stated previously, the habit of growth of the scion depends on the amount of living tissue in the roots of the rootstock. This relationship was established at East Malling (Beakbane and Thompson, loc. cit.) on the commercially useful rootstocks and was shown still more clearly by the examination of some of the most dwarfing types of rootstock produced by breeding at East Malling. The apple rootstock M. IX, used commercially, and the very dwarfing rootstocks 70 and 3426 gave a dwarf type of shoot growth in the natural unworked state. These extremely dwarfing rootstocks are very distinct from other forms that exist amongst apples and crab apples and it becomes important to ascertain if this dwarfing type occurs in any other genus of fruit trees. If this be so, it might then be of value to argue from analogy as to the effect on scions in other groups of rootstocks, or, alternatively, to assume that in the extreme types in other groups the scion habit will tend to conform to that of the natural shoot of the unworked rootstock.

The nature of the characteristics suitable for a biological study, as compared with those important for economic purposes, must be stated in order to define more exactly the scope of the present work. The economic value of an individual may be said to depend on small *quantitative* differences from other individuals in respect of a large number of attributes. In a biological study the attributes can more easily be examined if it is possible to concentrate on them individually, one at a time. This involves the use of the greatest possible *qualitative* differences to be found in the group. As it is desired to emphasize the need for obtaining as large a range as possible in variation for this approach to a rootstock problem, a more detailed description of the diagrams used in this paper is given as an Appendix (see page 131). This, it is hoped, may indicate more exactly how the direct observations on individuals can be applied to the selection of new material for experimental rootstocks. The suggestion is here made that even relatively crude methods of sorting material to be used in long term experiments are of use; and further it may be pointed out that the more the methods of sorting can be related to the biology of the individuals concerned the less crude the methods become. It is certain, however, that at present such methods cannot replace the long term experimental trials necessary for establishing really useful new rootstocks. Some of the main desirable properties of rootstocks are summarized below and, where possible, methods of determination which do not involve large scale field trials are indicated.

For the work described in the present paper the genus Prunus was chosen for study because *P. spinosa* and *P. domestica* occur wild and naturalized, respectively, in England.

Classification of the chief desirable qualities in rootstocks.

(i) *Propagation:* Ease of vegetative propagation is of first importance, because the use of clones is essential for any controlled experiments on rootstock properties.

(ii) *Working:* Considered as the initial union of stock and scion following budding and grafting.

(iii) *Vigour:* Dwarfing and invigoration of scion which may be correlated with precocity of flowering and fruiting.

(iv) *Compatibility:* Of rootstock and scion as concerned with the subsequent behaviour of the union between them. Compatibility is subject to varietal and species diverseness and, in general, is less frequent between species than within a single species.

The basis for separating (ii) and (iv) is that no general relationship between bud-take and compatibility is shown by the available data for the range of scions and rootstocks to be considered.

DETERMINATION OF THE DESIRED QUALITIES.

(*a*) *Indication by direct observations from nature.*

Propagation. In this paper two methods for examination of ease of vegetative propagation have been used : (i) observation of the rooting from sucker stems and (ii) small-scale cutting trials. The former can be observed directly in the field.

Ease of working. There are two indications relative to ease of working that can be observed directly : (i) the maintenance of continuous growth during the appropriate season for budding and (ii) the rapid formation of callus which may be essential in ensuring take of scions. The latter has not been tested by experiment but is indicated by the following observations : Quinces and most apples callus rapidly and are easy to bud ; plums, in general, form callus more slowly and are usually less easy to bud ; *Prunus nana* and *P. triloba* callus very badly and are extremely difficult to bud ; *P. spinosa* is intermediate between most plums and *P. nana* and *P. triloba.*

Vigour. The determining factors in the relation of structure to vegetative vigour and to precocity of fruiting were found to be the total living matter in the whole root together with the effective area of the vessels in transverse section (Beakbane and Thompson, 1939 ; Beakbane, 1941 ; Beakbane, Thompson and Tydeman, 1941). The total living matter in apple roots depends mainly on the per cent. bark together with the structure of the wood, because a relatively small amount of the bark is composed of fibres. Per cent. bark has, therefore, been used in the present preliminary survey because it accounts for most of the living matter and is a convenient figure to determine by measurements. Determinations of total living matter rather than simply per cent. bark would, however, increase the accuracy of the method of prediction. The total living matter would be most easily determined by chemical or biochemical methods. The relative amounts of haematin derivatives in the wood and the bark of apple rootstocks can be determined rapidly by a spectroscopic method, and the estimation of haematin is the subject of a separate investigation which will have a bearing on the present discussion.

Some of the differences observed in root structure are shown in the stem also, but to a much less extent. For example, the percentages of living matter in the wood of apple stems give a definite indication of vigour when the extremes of the range are compared. No significant differences in per cent. bark, however, could be detected in the normal range of apple rootstock stems. These results indicate that, for the present, the root is a more satisfactory part of the plant to examine than the stem when forecasting vigour. Attention has been directed to the structure of stems in relation to the properties of timber, but work on the root systems of plants in their natural habitat has not reached the same stage of development. One aspect that

limits the rapid progress of such work, namely the difficulty of obtaining roots, can best be illustrated by the following quotation from "The Systematic Anatomy of the Dicotyledons" by Hans Solereder (1908): "The structure of the root in the orders of dicotyledons has not yet been methodically investigated to any considerable extent, because the requisite material is generally wanting, and the anatomical investigation of the leaf and axis, which are more easily obtained and show a greater diversity of structural features, still affords abundant scope for research." This statement made in 1908, concerning the lack of methodical investigation of the structure of the root in the orders of dicotyledons, can still be applied to the present position.

(*b*) *Indication only by nursery and field trials.*

Compatibility of rootstock and scion: At present there is no method of estimating scion compatibility without actual tests involving working the rootstocks and making observations over a period of years. Scion compatibility, as usually understood, can be considered under two main headings: (1) the initial take of the scion, which is here classified under ease of working, and (2) the degree of scion-stock union that ensues during the growth of the tree. Work on the structure of the graft unions in cases of incompatibility suggests that this can conveniently be divided into three types: (i) clean-breaking type, (ii) discontinuous but partial union, (iii) a type that can be observed only by the behaviour of the scion. The clean-breaking type may not be evident at all in the growth of the scion, while a less obvious form of faulty union may be present when the scion makes poor growth or actually dies. This last state of incompatibility may be accompanied by extreme precocity of flowering. The relationship between precocity of bearing of scion and high per cent. of root bark of the stock, as shown by the apple, would not necessarily apply where the third type of incompatibility exists. This third form of incompatibility can readily be distinguished from compatible dwarfing, however, by the unhealthy appearance of the leaves, the lack of vegetative growth and the small size of the fruit. With compatible dwarfing the sizes of the leaves and fruits are not adversely affected. Compatibility may on the one hand extend to several related species, or it may be limited, even within a species, and at present there are no definite guiding principles; even that of close botanical relationship is known not to hold in certain cases.

Range of material examined and observations made.

The material represented by the wild and naturalized species *Prunus spinosa*, *P. insititia* and *P. domestica* was obtained from hedgerows in parts of Cambridgeshire, Kent and South Worcestershire. The three species of Prunus when growing under these conditions may seem when examined to form a nearly continuous range. The grouping adopted here is purely arbitrary, based as it is on leaf characters and habit of growth alone, for of many forms flowers have not yet been collected. Type of leaf serration, leaf shape, and presence or absence of thorns were the main characteristics used. Observations on the relative times of unfolding of the leaves and opening of the flowers were made. It is probable that some individuals included under *P. spinosa* will be found to be intermediate between this species and *P. domestica* or *P. insititia*. In Cambridgeshire, *P. insititia* is not so well defined as it is on the continent of Europe. The individuals grouped here under *P. domestica* would probably be placed in that group on any system of classification.

In addition to these wild and naturalized plums, some individuals of exotic species of Prunus cultivated in Britain were examined; for example, *P. triloba*, *P. nana*, *P. tomentosa* and *P. besseyi*. The specimens of *P. cerasifera* were collected in Cambridge from a hedge which probably consisted of seedlings imported from France.

Both mature fruiting plants and suckers of *Prunus spinosa* may be found growing in untrimmed hedges, and they sometimes form a scrub (Plate I, Fig. 1). In the ecological succession of plants on derelict cultivated land *P. spinosa* normally occurs after the hawthorn (*Crataegus oxyacantha*) has become established. In order to become established under natural conditions the seedlings seem to require the shelter of other bushes or trees. In hawthorn hedgerows the conditions are probably favourable for the establishment of seedlings of Prunus (Plate I, Figs. 3 and 4). In the hedgerow it is often possible to distinguish essentially different interpenetrating clones of *P. spinosa* suckers (Plate II, Fig. 6). The roots belonging to each clone can also be differentiated by their per cent. bark, size of vessels and amount of medullary ray tissue in the wood.

Most of the roots examined were taken from hedges since plants are more accessible to observation in a hedgerow than in a thicket. There is the further advantage of occasional ditching which exposes the roots and makes collection easier. Hedges are sometimes renewed by planting plum suckers along them and this may lead to the establishment of what appear to be extensive clones (Plate I, Fig. 2). Naturalized plants of the *domestica* type seem to have arisen from seedlings under the same conditions as those of *P. spinosa*. Chance seedlings of *P. cerasifera* have not yet been observed although this species is known to be used as a hedge plant in some parts of England.

Observations were made on rooting from cuttings and from sucker stems, and also on per cent. root bark in *P. spinosa*, *P. cerasifera* and naturalized forms of *P. domestica*.

VARIATION IN EASE OF PROPAGATION BY CUTTINGS.

Emphasis should be laid here on ease of vegetative propagation as being more important from the research point of view than for the grower, because it is possible to establish experimental results on compatibility and other indirectly observable characters only when very extensive clones are used. Vegetative propagation would remain, however, the most reliable commercial method until pure lines could be established. Ease of vegetative propagation of a rootstock from suckers, layers, root cuttings or stem cuttings depends in each case on the production of roots from the stem. The capacity for rooting from stems can be observed directly in wild plants when suckers are produced (Diagrams 1 and 2 and Plate II, Fig. 5).

In the individuals grouped here under *P. spinosa* it was found that rooting from stems was conspicuously rare, and when it did occur it was from two-year-old or older wood. In the material grouped under *P. domestica* from the Cambridge district, rooting from stems was common, and sometimes took place from the current year's wood. A selection of each type was tested for propagation by hardwood cuttings proceeding in exactly the same way as described by Garner (1944) for Myrobolan B. Thirty similar cuttings were taken in the autumn and planted in the usual way along with 30 Myrobolan B. In addition, 30 cuttings of *P. triloba*

Diagram 1.

Root sucker of *Prunus domestica* illustrating roots arising from the stem below ground level to show type of rooting from a one-year-old stem ideal for establishing an independent plant.

Diagram 2.

Root sucker of *Prunus spinosa* with no roots arising from the stem.

(which is known not to root easily from hardwood cuttings) were planted. Only two sets of wild plum cuttings rooted well and both belonged to the *domestica* type. These compared very favourably in growth with Myrobolan B. The rooting for two series of cuttings planted in October, 1944, and October, 1945 and examined in August, 1945 and August, 1946, respectively is shown in Table I.

The first series of cuttings observed to be making growth in August, 1945, had made good plants about 4 ft. high at the end of 1946.

Method of determining per cent. root bark.

Measurements of root bark were made with sliding callipers on fresh material. The diameter of the root was measured in two directions at right angles to one another. Cylinders of bark were then removed at suitable intervals from the measured region by making two cuts around the root to reach the cambium to allow measurements of the diameter of the wood to be made (Diagram 3). In winter it is necessary to soak the roots in water for a short time at room temperature to ensure easy separation of bark and wood at the cambial layer. It was found that prolonged soaking in water after partial drying may cause the bark to swell to a variable extent.

TABLE I.

Rooting of hardwood cuttings of wild and naturalized plums compared with those of Myrobolan B and Prunus triloba, *1945 and 1946.*

Reference number.	Type.	No. of cuttings rooted out of 30. 1944-1945.	1945-1946.
5	Gage	30	21
20	,,	7	—
4	*P. domestica*	23	12
21	*P. spinosa*	6	—
9	,,	1	1
34	*P. domestica*	8	—
H	*P. spinosa*	1	—
A	,,	1	—
Myrobolan B.	*P. cerasifera*	22	21
P. triloba B.	*P. triloba flore pleno*	0	—
43	*P. domestica*	—	13
W 8	*P. domestica* (Warwickshire Drooper)	—	19
L 5	*P. spinosa*	—	0
K 4	,,	—	7
K 1	*P. domestica*	—	8

The per cent. bark in a sample of roots is most conveniently estimated from the measurements as follows: If A and B=two diameters of the whole root taken at right angles to each other, and a and b=two diameters of the wood in the same directions, then the per cent. bark is given by $100 - \frac{100\,ab}{AB}$

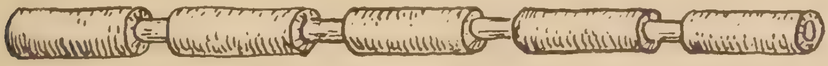

DIAGRAM 3.
Illustrating how the woody cylinder is exposed in order to ascertain its diameter.

RELATION OF ROOT THICKNESS TO PER CENT. BARK.

Measurements of individual roots from clones showed that the per cent. bark depends on the thickness of the root (Diagram 4). In no case were measurements of the white roots, i.e. those in which the true cortex had not been sloughed off, included. The decrease in per cent. bark with increasing thickness of root was found to be small when the percentage at 1 cm. thickness of root was above 65. When per cent. root bark is plotted against root thickness for a series of individuals the points of the observations seem to fall on a family of curves originating at a point which represents 75 per cent. bark between 0 and 1 mm. root thickness (Diagrams 5 and 6). A root having 75 per cent. bark has an equal thickness of wood and bark measured radially, i.e. the thickness of wood plus bark is twice the thickness of the wood alone. The most convenient root thickness for comparison was found to be about 1 cm. The variation of per cent. bark within the same range of root thickness

in roots taken from the same clone is shown for four different individuals in Diagram 8. The variation in each individual in this case seems to be due to two main factors: roots of different ages may be included in the same thickness range, and in Prunus the roots growing very near the surface tend to have a low per cent. bark. These factors will form the subject of a separate investigation. In the roots of *P. spinosa* annual rings in the xylem cannot at present be determined, and a quantitative description of the effect of root position on per cent. bark can best be based on individuals under cultivation. For separating the more extreme types the variation

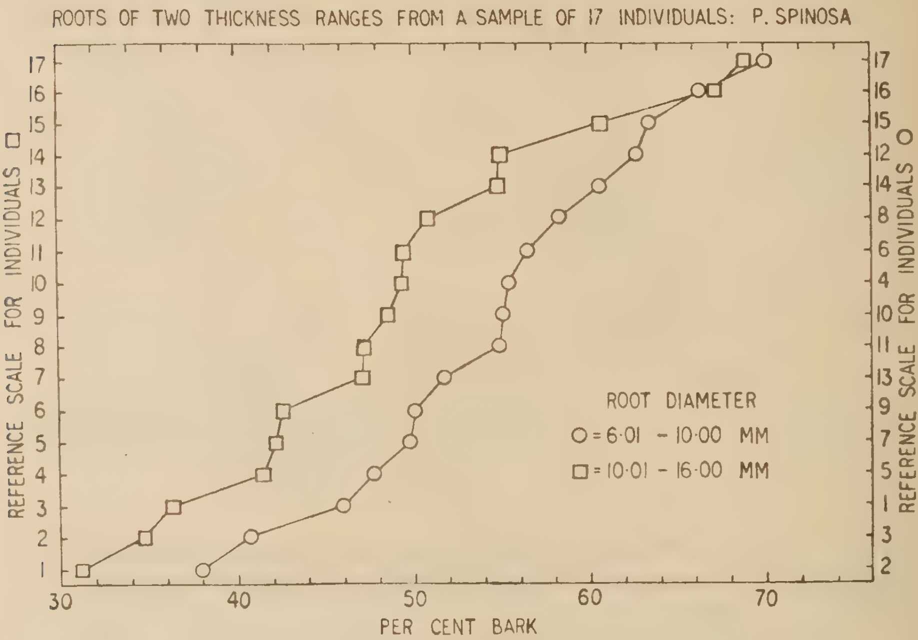

Diagram 4.

Per cent. bark of roots at two size ranges in seventeen individuals of *Prunus spinosa*.

in roots from the same tree has been reduced by selecting healthy roots from not too near the surface.

Variation observed in per cent. root bark of *Prunus spinosa*.

The range of variation found by measuring roots from individuals of *P. spinosa* is shown in Diagram 7 together with a similar group of data for *P. cerasifera* for comparison. For the latter the figure is derived from one measurement of a root, within the specified range of thickness, from each individual, and for *P. spinosa* it is based on the mean value of a varying number of observations on roots within the specified thickness range. The variation in per cent. bark of a number of roots from four individuals of *P. spinosa* is shown in Diagram 8.

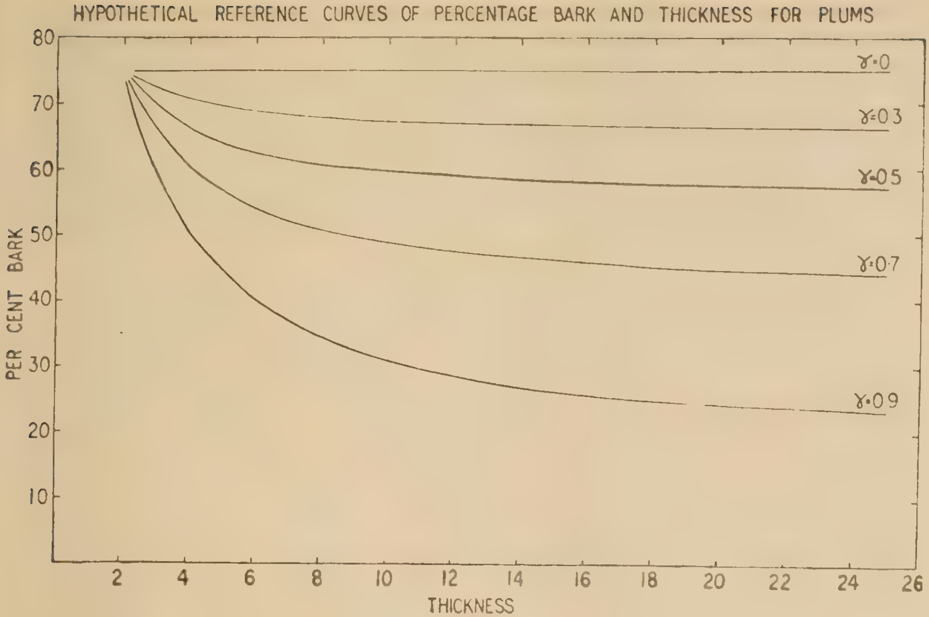

DIAGRAM 5.
Graph showing the relation of per cent. bark to thickness with five values of the constant γ plotted from the formula on page 132.

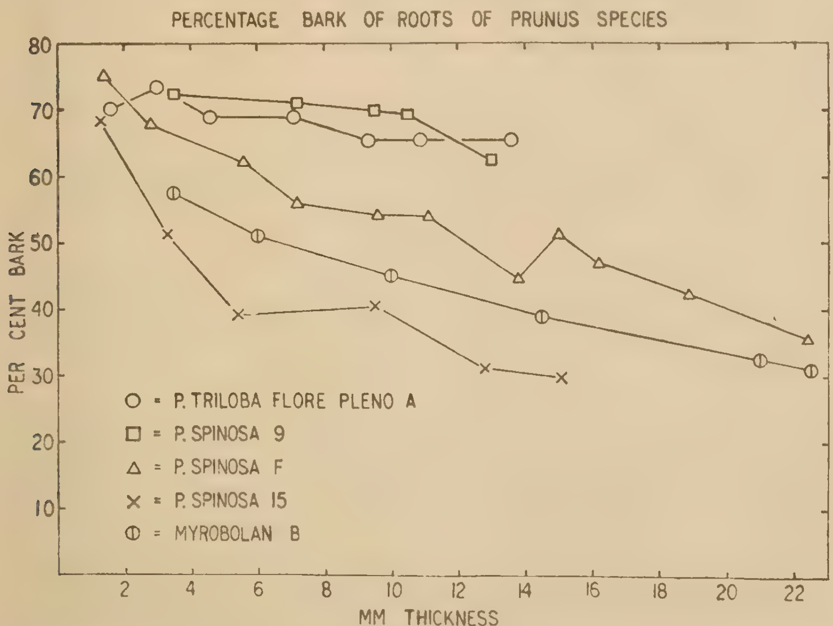

DIAGRAM 6.
Graph showing relation of thickness of root and percentage bark in individual Prunus.

The variation in per cent. bark as between different individuals (Diagram 7) is slightly greater than that of the samples of roots from a single individual (Diagram 8). It appears that the range of per cent. bark in *P. spinosa* is rather greater than that obtained by measuring roots of the commercial varieties of plum rootstocks. It is not possible to draw any fine distinctions between varieties of *P. spinosa* grouped in this way, but the data suggest that three groups of the species *P. spinosa* might be set up having approximately 40, 50 and 60 per cent. bark. The usual plum rootstocks lie within the range 40-50 per cent. bark for roots of comparable thickness.

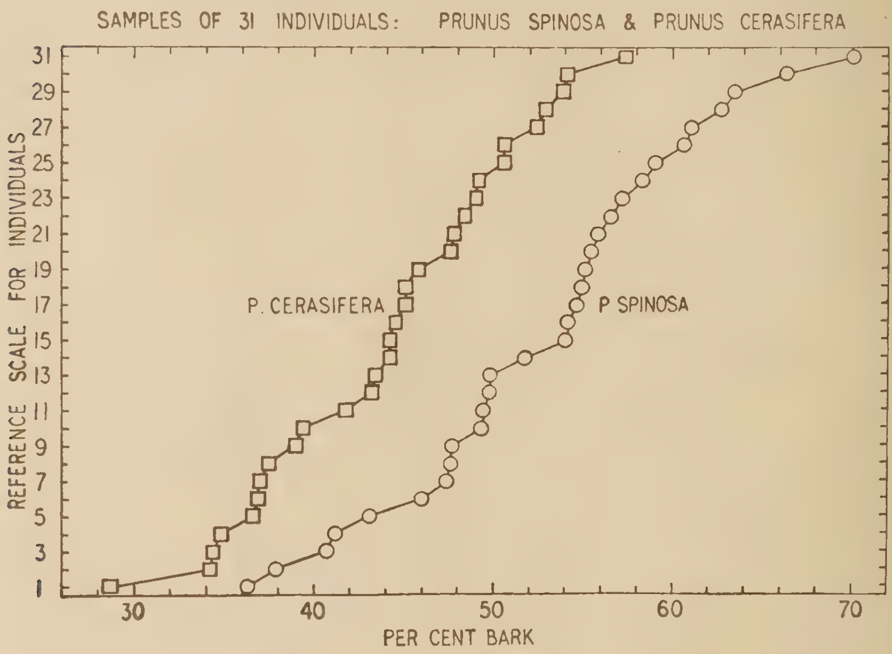

DIAGRAM 7.

Variation in per cent. bark between samples of thirty-one individuals of *Prunus spinosa* and of *Prunus cerasifera*. Roots 6-10 mm. diameter.

Referring now to the curve representing the data from *P. cerasifera*, not only is the range of variation less than in *P. spinosa*, but the mean per cent. bark from measurements of thirty-one individuals is less. Thus the species *P. cerasifera*, as represented by the sample selected, does not give any appreciable indication of individuals having a per cent. bark outside the range of that of the usual plum rootstocks.

When the relationship of per cent. bark to thickness of roots is studied it is seen that the variation of per cent. bark with root thickness is small in plants that have a high per cent. bark. In grading samples of roots it is thus possible to place more reliance on results obtained when the per cent. bark is high.

THE RELATIONSHIP BETWEEN PER CENT. ROOT BARK AND THE HABIT OF THE FREE-GROWING PLANTS.

Observations on unworked apple rootstocks M.I, II, IV, VII, IX, and XVI showed that while (with the exception of M. IV) they all made good growth at first, the stems of dwarfing rootstocks with a high per cent. root bark soon produced fruit buds and then differed greatly in habit from the more vigorous types, this effect being shown to a marked degree in the new clonal rootstocks 70 and 3426. Thus these unworked apple rootstocks, with one exception, show the same characteristics as the scions

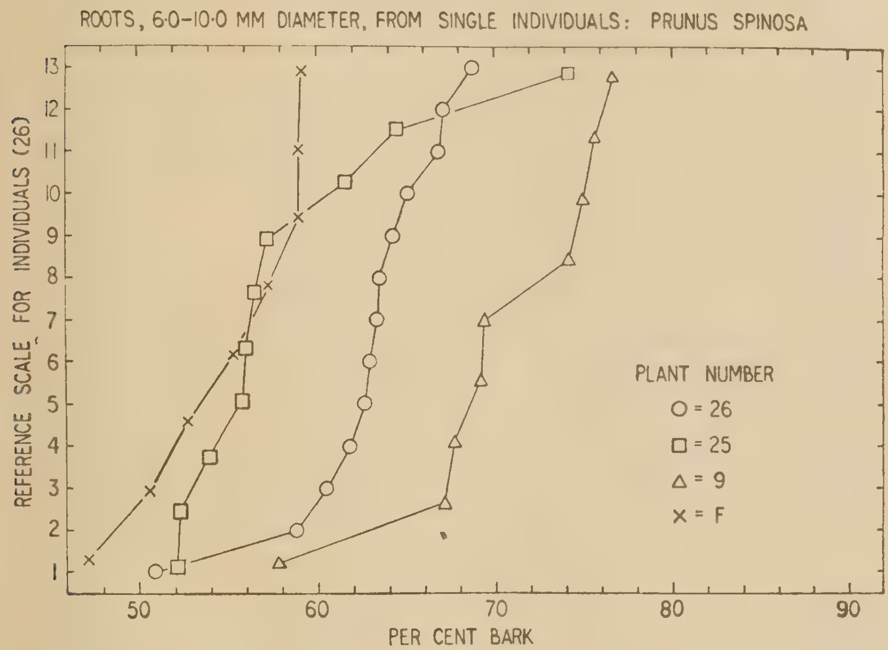

DIAGRAM 8.

Variation in per cent. bark in samples of roots from four individuals of *Prunus spinosa*.

worked on them. There is a large range in the genus Prunus as regards this type of dependence of vigour on early flowering (Plate I, Figs. 3 and 4, and Plate II, Figs. 7 and 8). If some of the species of Prunus are grouped according to their habit, as defined by relative size of top and relative precocity of flowering, and the per cent. bark of roots of similar thickness inserted, it is seen that high per cent. bark occurs mainly in the dwarfing group (Table II).

The dwarfing habit and precocity of *P. spinosa* and *P. glandulosa* is shown by the flowering of the relatively young suckers of *P. glandulosa* (Plate II, Fig. 7) and by the fruiting of the low bush of *P. spinosa* (Plate II, Fig. 8).

TABLE II.

The relationship between the per cent. bark of roots of some Prunus species and their habit of growth.

Vigorous.	% bark.	Thickness of root (mm.).	Intermediate.	% bark.	Thickness of root (mm.).	Dwarf.	% bark.	Thickness of root (mm.).
P. persica (Peach)	20	10				*P. nana*	65	10
P. cerasifera (Cherry plum)	40	10	*P. spinosa* (Sloe)	50	10	*P. glandulosa flore pleno*	80	8
P. avium (Sweet cherry)	37	7	*P. cerasus* (Acid cherry)	49	8	{ *P. besseyi* / *P. tomentosa*	65	8
P. domestica (Plum, Brompton-Malling clone)	46	10	*P. insititia* (Common Plum, Malling clone)	56	10	*P. triloba flore pleno* A	67	10

The range in per cent. root bark of *P. spinosa* extends almost to the known extremes of the vigorous and dwarfing types found in Prunus, as can be seen from Diagram 7. It is doubtful whether such a range of variation occurs in all groups; for example, per cent. root bark in *P. cerasifera* is shown to be less than in *P. spinosa* (Diagram 7). It is possible that the actual variation in per cent. root bark may be the same in both species, but as the mean for *P. cerasifera*, is lower than that for *P. spinosa* it is reasonable to expect that there will be fewer of the types with a high per cent. root bark.

DISCUSSION.

Taking as a type the unworked very dwarfing apple rootstocks, it has been shown that similar types occur in the genus Prunus as represented by *P. nana*, *P. triloba* and others. The species *P. spinosa*, as a whole, shows more tendency to this type of dwarfing characteristic than *P. cerasifera*, and the results presented suggest that by selection from *P. spinosa* comparatively dwarfing forms could be obtained. There is good evidence in apples and a little evidence from previous work with stone fruits, that in extreme types the scions behave similarly to the unworked shoots of the rootstocks. By including observations on root structure it would then be possible to distinguish the more dwarfing type of rootstock in groups other than the apple. Under cultivation the environment of a fruit tree is intermediate between that of the wood, supporting the vigorous type, and the open country, supporting the dwarfing type. This is shown in cultivated apples by interplanting with trees on a dwarfing type of rootstock which show a good performance over the first 10 years; these can then be removed and the more vigorous trees allowed to continue growing. It should be pointed out that some of the fruit varieties

themselves might be traced to a combined origin from these two types of environment. The domestic plum is considered to have resulted from hybridization between *P. spinosa*, a semi-dwarfing type and *P. cerasifera*, a more vigorous one (Crane and Lawrence, 1938). The apple varieties could be considered as resulting from hybrids representing the vigorous *Pyrus malus sylvestris* section and the more dwarfing *P. malus pumila* section of the species. The qualities here defined as dwarfing characteristics appear to be correlated with the herbaceous type of structure, which might have resulted from natural selection operating on plants having a shorter life cycle than that of the forest tree. It is clear, however, that in the maintenance of vigour the tree is dependent on dead tissues for mechanical support, thus showing that the structure of the vigorous tree would also be the result of natural selection.

In the artificial selection that has resulted in originating cultivated plants, breeding of new varieties continues mainly from already established forms. Suitable forms occurring spontaneously have also been used from ancient times for breeding, in addition to the established forms. It is considered that this may not have been so with fruit tree rootstocks. This point may be illustrated as follows: Extreme deviations from a species type may be rare, but when the deviation is in the direction of an improvement in either fruit or flower, it can often be recognized immediately by a relatively unskilled observer. With rootstocks, however, which require to be budded and subjected to long-term observation of the scion before their potentialities can be tested fully, many of the desirable qualities cannot be recognized immediately.

If four qualities were required to be obtained from individuals by a selection method, it is evident that the chances of getting them all in one individual are small. Assuming that there is a 10 per cent. chance of finding one of the qualities in an individual member of a wild species, the chances of combining the four qualities in one member would be 1 in 10,000. The problem of selection may be simplified by the study of variation in immediately observable characters. Knowledge of the magnitude of the variation is valuable in the elimination of unsuitable individuals and even of sub-populations of seedlings, or local associations of plants; but at present the range of variation in any of the previously mentioned characters has not been determined for plum varieties or rootstocks. It is probable, however, that plums such as Pershore or Gage, which come more or less true from seed, will show some variation in the characters mentioned above. For example, although the Malling clonal Pershore is relatively difficult to propagate vegetatively, Pershore plums as a whole most probably represent many clones and may include types which are easy to propagate. This could be determined by a study of the variation in rooting from stems, within the group.

Although breeding new forms would eventually be the most satisfactory method of producing a range of Prunus rootstocks to meet growers' needs, the collection of chance seedlings saves time in several ways, viz. (i) mature trees can be observed immediately and thus a period of delay extending from ten to twenty years for new seedlings to mature under cultivation can be avoided; (ii) the spontaneously arising seedlings must be selected in the direction of maintaining satisfactory growth and resistance to diseases such as Silver Leaf and Bacterial Canker, and (iii) in selections from the field, observations can be made directly *in situ* on such matters as the type of root system, the capacity for rooting from stems, the formation of callus tissue and the mechanical properties of the stem, again saving time in comparison with long-term breeding.

The chance seedlings that have been examined in the course of the present work showed great variation in per cent. root bark, rooting from stems, and other characters. In earlier work, Hatton (1921) observed variations in the vegetative vigour of *P. spinosa* seedlings. In describing the seedlings he says : " *Prunus spinosa*, though catalogued by some Continental firms in their lists of Plum stocks, is, I imagine, very little used at the present time. The collection I received proved to be one of seedlings, some of which were far more vigorous than others. Conjecture would lead one to suppose that some of these would be very dwarfing in effect." The present work bears out the surmise, since the range of variation in per cent. root bark in *P. spinosa* is very much greater than that exhibited by the usual plum rootstocks. It would thus be possible to select individuals from *P. spinosa* with a range in per cent. bark nearly equal to that existing between M. IX and M. XII apple rootstocks.

It has thus been indicated that the range of properties in the existing plum rootstocks classed as dwarfing action and ease of propagation from cuttings could be extended by selection from material already at hand. At the present time the possibilities of selection from naturally existing forms both in this country and abroad are very far from being exhausted, and investigation can continue of the existing range of variation both from the point of view of obtaining experimental material and also in preparation for the breeding of new plum rootstocks.

SUMMARY.

1. An attempt has been made to define some of the main desirable properties of fruit tree rootstocks.

2. The properties chosen for consideration are grouped as follows :—
   (1) indication by direct observations in the field on (i) ease of vegetative propagation, (ii) working, (iii) vigour ; and
   (2) indication by experimental trials of compatibility of rootstock and scion.

3. A description of apple rootstocks is given showing how the effect of the unworked rootstocks of extreme types on their own stems agrees in general with their effect on scions worked on them.

4. Observations have been made on wild and naturalized fruit trees which show the existence of a greater range of useful material than is usually available in ordinary horticultural practice.

5. Possible methods of recognition of properties in the field are discussed. This might result in the rapid recognition of good types for experimental trial.

6. Ease of vegetative propagation with field material was tested by growing cuttings.

7. The data on habit of growth in relation to amount of living tissue in roots obtained from the unworked extreme dwarfing types of apple rootstocks have been found to apply to certain species of Prunus.

8. When making use of the present hypothesis in estimating vigour it was found necessary to measure the variation of per cent. bark in relation to thickness of root.

APPENDIX.

Graphical representation of variation of measurements on individuals.

The method of grading originally introduced by Galton (1889) has been found to be the most convenient one for graphical representation of the measurements of per cent. bark in roots. The S-shaped curve is known as Galton's ogive or the summated frequency diagram. The diagram thus shows the individual observations directly and is not limited by the economy introduced by statistical treatment. Such statistics as are required can be read off graphically from the diagram with sufficient accuracy for this type of investigation. For exhibiting and discussing the information relative to different varieties the ogive diagram has been found more convenient than the frequency diagram. The results, therefore, are given in the form of these summated frequency curves, or ogives. The initial difficulty in the appreciation of the summated frequency diagram is the significance of the ordinates. Galton discusses this point in his original description in which he represents the ogive with the axes reversed in relation to x and y as compared with the diagrams in this paper in which the y axis represents simply the reference number of an individual (case) in its graded order. In the present work small samples of about 30 individuals are used. If the ogive is plotted directly from measurements, and the scale of reference amounts to one unit, the slope of the median portion of the curve is an estimation of $\frac{1}{\sqrt{2\pi}} \times$ standard deviation; while should the curve be appreciably symmetrical, the median portion is a good approximation to the mean. If necessary the curves can readily be fitted by the use of probits and, when advantageous, the logarithmic transformation is used. It should be mentioned, however, that the significance of the curves can be appreciated without any reference to statistics, and any marked departures of a range of grades from a possible fit rapidly recognized. This last statement was accidentally confirmed in two instances in which plums under cultivation were examined in relation to per cent. root bark. The ogive diagrams showed two definite points of inflection, and this was eventually explained by reference to records, which became available at a later date, showing that the rootstock plants were mixed.

Variation of per cent. bark with thickness of root in the same individual.

When a series of measurements of per cent. bark is made on roots from different plants and the per cent. bark is plotted against thickness of root, the series of curves shown in Diagram 6 is obtained. The per cent. bark in small roots in the species of Prunus examined always appears to tend to be 75. The change with root thickness may be rapid at first, but becomes less as the thickness increases. As the relation between per cent. bark and root thickness is not represented by a straight line it is to be concluded that the production of phloem relative to xylem varies during the growth of the roots. The whole of the root bark will at present be considered as phloem after the initial shedding of the cortex, and no measurements made before this takes place have been included. A study of all the data so far obtained suggests three assumptions which might account for the relation between per cent. bark and root thickness in the simplest way. The production of equal radial widths of phloem and xylem during a preliminary interval can be assumed and this can be followed by the assumption that in subsequent intervals, instead of equal thicknesses of phloem and

xylem being produced from the cambium, there will be a constant diminution in the amount of phloem. Lastly, there must be the assumption that the increase in a tangential direction outside the cambium takes place in such a way as to leave the radial thickness of bark unchanged. From these three assumptions it follows that the per cent. bark will gradually fall with increasing age, but will tend to approach a constant value. The less the production of phloem the more rapid will be the fall in per cent. bark with age, and the lower the final constant value reached. The following formula is presented to illustrate the consequences of the above assumptions.

Let θ = ratio: phloem/xylem (radial lengths) at end of first interval.
,, ϕ = ratio: phloem/xylem added during subsequent intervals.
,, β = diameter of xylem at end of first interval.
,, x = diameter of xylem greater than or equal to β.
Then total thickness of phloem at end of first interval $= \beta\theta$.
total thickness of root at end of first interval $= \beta(1+\theta)$.
Put A = total thickness for any value of x,

$$= \beta(1+\theta)+(x-\beta)\ (1+\phi).$$

$$\text{Then } x = \frac{A+\beta\ (1+\phi)-\beta(1+\theta)}{1+\phi}$$

$$\text{Put } \alpha = \frac{\text{phloem}+\text{xylem}}{\text{xylem}} \text{ at end of first interval} = 1+\theta.$$

Put $1-\gamma$ = proportion of phloem relative to xylem produced after the first interval.

$$\text{Then: } \gamma = \theta-\phi$$

$$1+\phi = \alpha-\gamma$$

$$x = \frac{A-\beta\gamma}{\alpha-\gamma}$$

$$\text{per cent. bark} = 100\left(1-\frac{x^2}{A^2}\right) = 100\left\{1-\frac{(A-\beta\gamma)^2}{A^2(\alpha-\gamma)^2}\right\}$$

Graphs with five values of γ are plotted in Diagram 5; here $\alpha = 2$ to accord with the present observations, and β is arbitrarily made equal to unity. The formula is derived from the simplest possible assumptions and is intended only as a guide to the interpretation and analysis of the results. The index γ will, on the present hypothesis, represent the relative vigour. This is in accord with the observation that the per cent. bark falls rapidly with increasing root thickness in the more vigorous apple rootstocks.

No attempt has been made to fit the curves to the results obtained because there remain two sources of error which must be considered. The first is that in plums, roots very near the surface may have a lower per cent. bark. The second source of error is that for a given root thickness range, roots of very different ages may be included, and this involves a study of the actual growth rates—not considered here. It is possible to determine this with some of the apple rootstocks, but in plums the root xylem does not show annual rings to any extent. By taking healthy roots at a moderate depth both these sources of error may be reduced. The data shown in Diagram 4 give a partial estimate of these sources of error. It is also seen here that the ogives tend to coincide at high per cent. root bark, and this is in accord with the method of representation illustrated by Diagram 5.

PLATE I.

FIG. 1.

Suckers of *Prunus spinosa* spreading into *Bromus erectus* sward, and flowering while still fairly small. On chalk soil near Wrotham, Kent. April 11th, 1946.

FIG. 2.

Large clone of Prunus No. 5, of *domestica* type, forming part of a field hedge in Cambridgeshire. April 9th, 1946.

FIG. 3.

Suckers of *Prunus spinosa* flowering while still young. At the edge of a greengage plantation in Cambridgeshire. April 10th, 1946.

FIG. 4.

Suckers of Prunus of *domestica* type. At the edge of the same plantation as in Fig. 3. April 10th, 1946.

[*face p.* 132

FIG. 5.

Sucker of Prunus No. 43 of *domestica* type, rooting from the stem at ground level. From a field hedge in Cambridgeshire. April 9th, 1946.

FIG. 6.

Suckers of two different types of *Prunus spinosa* growing from the hedge shown in Fig. 1. July 30th, 1945.

FIG. 7.

Sucker of *Prunus glandulosa flore pleno* flowering one year after planting. At East Malling Research Station. April 27th, 1946.

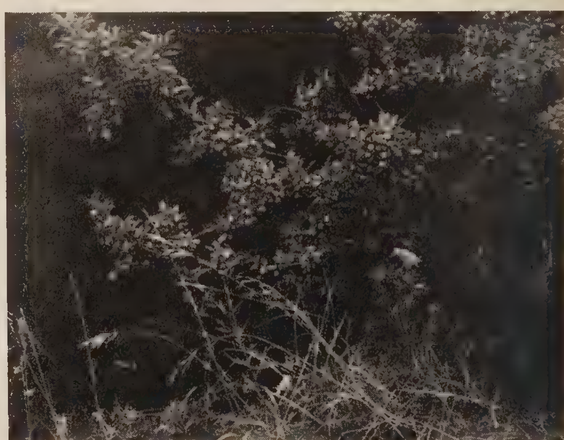

FIG. 8.

A bush of *Prunus spinosa* showing medium dwarf habit and precocious fruiting. Near Wrotham, Kent. July 30th, 1945.

ACKNOWLEDGMENTS.

The authors wish to thank Dr. R. G. Hatton and Professor A. C. Chibnall for helping to arrange the collaboration between them in connection with this work. They would like to thank Mr. S. C. Pearce for many helpful suggestions concerning the explanation of the diagrams used. They are grateful to Professor F. T. Brooks for providing facilities in his Department, and to many friends who have helped with the collection of material. The photographs reproduced in Plates I and II were taken by Miss E. C. Thompson.

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PRELIMINARY EXPERIMENTS ON THE INJECTION OF INDIVIDUAL APPLE FRUITS ON THE TREES

By B. F. G. LEVY
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INTRODUCTION.

Plant injection has been used successfully for studying the effect of nutrient substances on fruits while still on the tree, e.g. by Atkinson (1935), Hulme (1937) and others. In this previous work, the injections were carried out on whole trees or on individual branches ; but for some purposes a much smaller unit would be preferable. Investigations were therefore made on the possibility of injecting individual fruits on the tree.

INJECTION OF THE WHOLE FRUIT.

Where a single fruit is borne on a spur, there is often a current year leafy shoot growing on the same spur system not far below the bourse (Fig. 1), and it was found that injection of the fruit could be carried out through such a shoot. One was chosen of sufficient length (about 2 inches) to be bent over, and its cut end was plunged below the surface of a solution contained in a small vessel.

In trial injections with dye solutions, the rate of absorption was good, about 10 ml. being taken up within 2 or 3 hours. Spurs so treated were subsequently

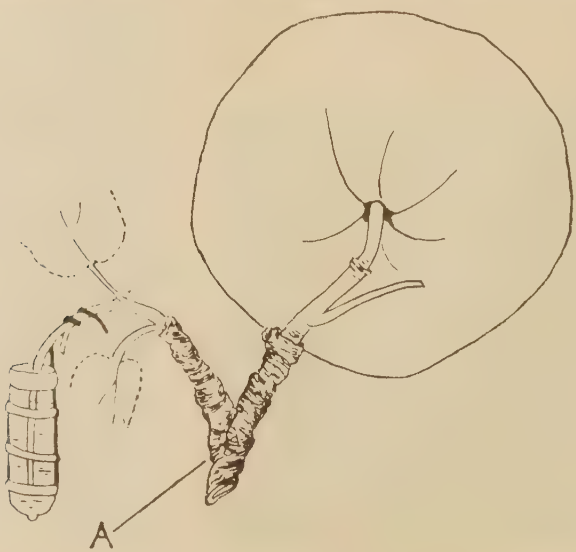

FIG. 1.
Whole fruit injection.

cut off and dissected, so that the course of the dye could be followed. It was seen that most of the spur system had become permeated, although the dye did not travel very far below the point of origin of the injected spur (A, Fig. 1). The actual distance varied with the number of leaves on the spur below this point, but a large proportion of the dye travelled up into the bourse that bore the fruit, whence it travelled into the fruit stalk, which became distinctly dyed, the colour becoming visible from the outside.

When the fruit was cut across equatorially all of the ten vascular bundles were seen to be well dyed, and the colour had radiated out from all of them, suffusing the flesh. The depth of colour of the dye diminished towards the margin of the apple until, at about $\frac{3}{4}$ inch from the skin, it could no longer be seen with certainty.

When there were two or more apples on the same bourse, then that fruit which arose nearest to the injected shoot became fully injected, as described above, but the other fruit or fruits became only partially injected. When these other apples were cut across equatorially, only one or two of the vascular bundles in each fruit were seen to be dyed, namely, those on the side towards the injected shoot. The exact distribution of the dye in these bundles seemed to depend on the position of the fruit on the bourse. In view of the difficulty of following the phyllotaxy after all unfertilized blossoms have fallen, the position of the injected sector could hardly be predicted, consequently another method for deliberate partial injection seemed desirable.

PARTIAL INJECTION OF A FRUIT.

Further injections revealed that for partial (sectorial) injection, a fruit that had a secondary (current year) shoot arising adjacent to it on the same bourse (Fig. 2) was suitable. This shoot was bent over and injected as was done for the whole fruit injection. When this shoot was not long enough, injection was carried out through

FIG. 2.
Partial fruit injection.

the petiole of a leaf borne on it. The cut petiole was bent over into a small receptacle attached to the shoot by a rubber band or by lead wire.

On dissection, it was found that the effect of this procedure was that the dye had travelled into the bourse and then straight up into the apple stalk, hardly any of it going down into the main spur system. When there was more than one apple on the same bourse, then the dye travelled only into that fruit, the stalk of which was closest to the secondary shoot.

The apple stalk, however, became only partially injected, only those vascular bundles on the side nearest the injected shoot becoming dyed. The result of this could be seen when the fruit was cut equatorially; the dye had been injected into only one or two of the five main vascular bundles, and only that part of the flesh was dyed which was supplied by these bundles.

Discussion.

Fruit injections by the first method make possible the study of the effect of a number of different substances (or different concentrations of the same substance) on a disorder such as Corky Core, by injecting each into single fruits on different spurs on one and the same branch. Thus, a small experiment could be carried out on a single branch of a tree and a larger one on a single tree. In this way tree to tree variation (which is often marked in trees affected by mineral deficiency) could be avoided. Further, in plantations in which trees show symptoms of such deficiency the extreme manifestation of the disorder is often seen in only a few of them, and they would be of particular value for experimentation. By single fruit injection, efficient use could then be made of material which would be unsuitable for experimentation by most other methods.

SUMMARY.

In preliminary experiments with dye solutions individual apple fruits were injected whilst on the tree.

Injection through the cut end of a leafy shoot arising near the base of a bourse bearing a fruit resulted in total permeation of that fruit.

Injection through a secondary shoot arising on the same bourse as the fruit, resulted in the permeation of one or two sectors of the fruit only.

The injection of nutrient solutions by either of these methods should prove of use in the study of physiological disorders in trees in the field.

ACKNOWLEDGMENT.

The writer is much indebted to Dr. W. A. Roach for valuable help.

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BOOK REVIEWS

THE DESIGN OF EXPERIMENTS. By R. A. FISHER, Sc.D., F.R.S. (Edinburgh and London: Oliver & Boyd. Fourth edition, 1947. Price 12s. 6d.)

Earlier editions of this work have played an important part in spreading a knowledge of the fundamental principles of experimental design, so many of which are due to Professor Fisher himself. The latest edition follows the same pattern as its predecessors, and does not aspire solely to inform the reader what is the correct design to meet every situation, but stresses the principles upon which his decision should be based. The merits of the principal types of design—randomized blocks, Latin squares, factorial and confounded factorial designs—are also set forth in detail, and worked examples are given which will be of great assistance in understanding their intricacies.

These examples are mainly taken from agricultural practice, and care must be taken not to apply all the designs to horticultural experimentation without considering its special problems. In this connection it is rather unfortunate that only a short reference is made to the possibility of missing plots, and it is suggested that they need cause little trouble in the interpretation of complex experiments. This is less likely to be so with perennial material, and there is a danger that a number of missing plots will completely ruin an involved design.

The basis of methods of improving the accuracy of comparisons by correcting the records of an experiment by some other quantity—such as crop over a period before experimental treatments have been applied—is lucidly explained. This section will be of particular value in pomological work, in which the high variability of experimental material is liable to make comparisons not very precise, but corrections for earlier crops or measures of vigour may reduce the errors appreciably.

Professor Fisher's book remains indispensable to all concerned in the designing of trials. A great part of it, and in particular the earlier chapters, may be read with much profit by those chiefly interested in the interpretation of experimental results.

J.T.

MARKET GARDENING AS A CAREER. By J. O. BAKER. (Vawser & Wiles, Ltd., 1946, 64 pp., 8 pp. Plates. Price 5s. net.)

This is an excellent little work for young people entering on a horticultural career, or for ex-Service people thinking of taking up a small-holding. It deals with useful matters, viz.:

1. The Land Settlement Association.
2. Renting or Buying Land.
3. Capital required for a 10-acre, down to a 2-acre, intensive holding.

Mechanization in the small market garden is fully dealt with.

The suggested plans for cropping for different purposes, together with average returns, are also helpful.

In the Appendix information is given on the training given at Schools, Colleges, etc.

Information concerning Advisory Services, lists of books, and many useful tables are also supplied.

H.C.C.

ARTIFICIAL ILLUMINATION IN HORTICULTURE. Critical Résumé. By DOROTHY BRANDON. (Technical Report (Reference W/T 11) British Electrical and Allied Industries Research Association. London, 1946. 17 pp. 4s.)

Scientific and practical horticulturists will welcome the publication of this Report which gives, in an admirably concise form, an up-to-date account of experimental work dealing with the growth of plants under conditions of artificial illumination. The main emphasis is laid on the control of day-length, and the possibilities it offers for the forcing of glasshouse crops, though many other aspects of the problem, such as the effects of light intensity and light quality, are also considered. For the benefit of those unfamiliar with the principles of plant physiology the Report opens with a simple account of the chief vital processes in the plant that are influenced by light. A chronological survey of research work on the subject of plant irradiation follows, and then a review of the use made of artificial illumination in other countries is given. The need for much further information concerning the light requirements of British glasshouse crops is stressed, and, to the research worker in this field, guidance on the choice of lighting equipment is given. A valuable feature of the Report is the comprehensive list of references.

L. C. LUCKWILL.

OCCASIONAL PUBLICATION ON SCIENTIFIC HORTICULTURE. No. 5 (March, 1947), pp. 96. Published by the Horticultural Education Association. (Obtainable from Gibbs & Sons, 16 Orange Street, Canterbury. 4s. 3d. post free.)

This brings to an end the series of Occasional Papers published by the Horticultural Education Association as a wartime measure. It contains short useful surveys by the leading English authorities of subjects which have been much in the minds of British horticulturists in recent years. Four papers concern mineral deficiency problems and methods of investigation. Advances in the use of growth substances are noted in a fifth. A paper on field ploughing is followed by one on recent advances in horticultural machinery of different kinds and a third on spraying machinery. The position with regard to bacterial canker and leaf spot of plum and cherry is set out and other more general articles concern control of vegetable diseases, DDT and other insecticides, and pests of brassica seedlings. Most of the articles give a clear outline of their subject and provide references to original articles for those who want to study the matter further.

D. AKENHEAD.

BOOKS RECEIVED

JOURNAL OF THE INSTITUTE OF CORN AND AGRICULTURAL MERCHANTS, LIMITED. (W. Heffer and Sons, Ltd., Cambridge, April 1947, pp. 40, 10 plates. Price 5s.)

LAND CLASSIFICATION IN THE WEST MIDLAND REGION. Published by the West Midland Group on Post-war Reconstruction and Planning. (Faber and Faber, Ltd., London, W.C.1. 1947, pp. 48. Price 12s. 6d.)

Dr. G. H. Pethybridge.

[frontispiece

Volume xxiii is Dedicated to
Dr. G. H. PETHYBRIDGE,
Assistant Editor,
1936-1947.

EDITORIAL

At the last meeting of the Editors and the Publication Committee, we had to report with much regret that Dr. G. H. Pethybridge, who had acted as Assistant Editor since (late) 1936, felt that he could no longer undertake this detailed work.

Very few people will realize what this work has entailed and how much the *Journal* has owed in the past ten years to his fearless and constructive criticism of manuscripts and to his unremitting attention to detail, which would make a paper at once more concise and intelligible. Over the period in which he has acted in the capacity of Assistant Editor, he has immeasurably improved the standard of the presentation of the papers which have appeared.

The Editors and Publication Committee decided that the most appropriate way in which they could express the *Journal's* indebtedness to him was by dedicating this Volume to him and by including his portrait. All those who know Dr. Pethybridge personally will realize how difficult it has been to secure his consent to these proposals.

This is not the place to dwell upon his distinguished services at the Ministry of Agriculture's Plant Pathology Laboratory, Harpenden, but rather to record our good fortune in securing his assistance on his retirement from that post. Not only are those responsible for the production of the *Journal* grateful for all the invaluable work he has unsparingly undertaken, but also the contributors of manuscripts have realized the great value to them of the criticisms and suggestions which he has put forward, always with an open mind.

We are happy to say that Dr. Pethybridge has consented to maintain his contact with the *Journal* as an Associate Editor, although he can no longer undertake the more laborious duties, which he has performed so successfully.

In the Editorial which appeared in May, 1947, in Parts 1 and 2 of Volume XXIII, the hope was expressed that it might be possible to issue that Volume of *The Journal of Pomology and Horticultural Science* during the year, and to start 1948 with Volume XXIV under the simplified title of *The Journal of Horticultural Science*. The Editors still hope to return to normal and to issue four parts of the *Journal* in a calendar year.

R.G.H.
T.W.

IMPROVING THE FIELD PERFORMANCE OF STANDARD PROTECTIVE FUNGICIDES

II. THE USE OF FERROUS SULPHATE TO DIMINISH SPRAY DAMAGE ON APPLE

By M. H. MOORE
East Malling Research Station, Kent

The chief drawback of lime-sulphur is its well-known property of causing leaf- and fruit-drop in summer, especially on certain apple varieties, notably the commercially popular Cox's Orange Pippin. This applies not only to the fungicide itself but perhaps even more so to its combination with arsenical sprays. Some satisfactory means of obviating this might well enhance its prominence as a safeguard against certain diseases, particularly Apple Scab (*Venturia inaequalis*), for in other respects it combines many of the attributes of the ideal protective fungicide.

According to Martin (1940), much of the relevant literature suggests that sulphides in solution are most likely responsible for lime-sulphur damage, a view that he (1930) was unable to support as regards leaf-drop on gooseberries. The considerable diversity of opinion on this matter may derive from there being two distinct kinds of damage, leaf-scorch and leaf-drop (often with no sign of scorching), probably brought about in different ways. Berry (1938) inclined to this view and suggested that leaf-drop may be caused by hydrogen sulphide formed by hydrolysis of the monosulphide part of the soluble sulphides (Martin, 1940, p. 116) as well as by enzyme activity when sulphur is in contact with living plant tissue. Precipitation of the monosulphide by the introduction of certain metallic sulphates, e.g. the ferrous salt, before spraying would appear to offer a simple way out of the first difficulty. In the lime-sulphur-lead arsenate mixture, phytotoxic soluble arsenicals are formed as reaction products from the hydrogen sulphide and the arsenate, and prior precipitation of the monosulphide with a metallic sulphate should solve this problem also (Martin, 1940). On theoretical grounds (Kearns, Marsh, and Martin, 1934), the inclusion of a metallic (in this case, ferrous) sulphate should improve rather than impair the fungicidal value of the spray, for the monosulphide is no longer lost as hydrogen sulphide, but ultimately becomes free sulphur available for fungicidal action.

This lime-sulphur-ferrous sulphate mixture has been known for many years, for Salmon (1912) mentioned that self-boiled lime-sulphur with ferrous sulphate was very adherent and safe to use on Warner's King apple trees.

In commerical practice various metallic sulphates have received attention in the Eastern Provinces of Canada and the U.S.A., largely owing to the work of Kelsall and Hockey in Nova Scotia. Kelsall, Hockey, and Walker (1930) published a review of six years' experiments in which they tested zinc sulphate, aluminium sulphate, and ferrous sulphate respectively. The zinc-sulphate mixture was not promising as a fungicide. The aluminium-sulphate one, with calcium arsenate as a stomach poison, proved to be a safe, efficient fungicide and insecticide, but corroded the pump valves and seatings and produced much poisonous hydrogen sulphide in the spray tank. The ferrous-sulphate mixture was the most satisfactory in all respects and ultimately (Hockey, 1931; Kelsall, 1935) became established in the

routine spray programme. Calcium arsenate was preferred to lead arsenate because the mixture was less phytotoxic and more fungicidal.

Folsom (1933, p. 491) stated that "in 1932, on bearing McIntosh trees, the addition of iron sulphate to lime-sulphur in the 2-week, 4-week and 6-week applications apparently gave a definite decrease of leaf injury, a definite increase of yield, and favourable effects in other particulars." Erni (1931) reported satisfactory results from lime-sulphur with ferrous sulphate in Switzerland; and other, similar, references can be found in the literature.

There were thus prospects that the addition of commercial ferrous sulphate to lime-sulphur sprays would obviate much of their phytotoxicity, which is common in England, particularly the south-east. The results of the present experiments have already been summarized briefly for fruit-growers from time to time (M[oore], 1936-38).

THE EXPERIMENTS.

Two-year-old Cox's Orange Pippin and Worcester Pearmain trees, forty-one of each variety, on No. II rootstock, were planted alternately at 12 ft. apart in three rows in the winter of 1930-31. Two of the Worcester trees died early and were not replaced.

While the trees were becoming established, some preliminary data were obtained on them by comparing lime-sulphur plus lead-arsenate paste with lime-sulphur plus calcium-arsenate powder, each with and without the addition of ferrous sulphate. The calcium-arsenate spray was the more phytotoxic; it caused severe leaf- and fruit-drop, especially on Cox. Ferrous sulphate mitigated but, especially as regards fruit-drop, did not eliminate this damage. The ferrous sprays imparted a darker green colour to the leaves (probably through iron absorption) and, black when applied, proved very persistent as a conspicuous, rusty deposit.

1935-36 Trial.

With the above information as a basis, a new trial was planned on the same trees in 1935. Seven treatments (Table I), ten trees to each, were compared; they were randomized separately on each variety in five replicated blocks, i.e. fourteen trees (two varieties) per block, leaving ten spare trees, six of Cox and four of Worcester, mostly on the headlands. On the last-named ten an eighth treatment was included to test the effect of zinc sulphate instead of ferrous sulphate.

SPRAYS.

1935. Lime-sulphur (l-s.) was applied at 1-40 v/v pre- and 1-60 post-blossom (to ensure spray damage); calcium-arsenate powder (c.a.) and lead-arsenate paste (l.a.) at 0·4% w/v pre- and post-blossom; and ferrous sulphate (f.s.) and zinc sulphate (z.s.) pre- and post-blossom at 4 lb. crystals to every gallon of lime-sulphur concentrate, irrespective of dilution (Kelsall *et al.*, *loc. cit.*).

Nicotine (0·05% w/v) and a wetter* was applied to all trees, controls included, at Petal Fall against Sawfly; on treated trees it was included in the Petal Fall spray.

The sprays were applied fairly heavily at 250-300 lb. pressure per sq. in., either from a hand-pumped "Cascade" machine or from a small, mobile power sprayer. Applications were made at the Pink Bud (April 30th), Petal Fall (May 23rd), and Fruitlet (June 17th) stages.

1936. All trees, controls included, received two lime-sulphur (1-40) and lead arsenate (0·4%) sprays pre-blossom (April 24th and May 6th), and the differential spraying as above was continued at the Petal Fall (May 28th) and Fruitlet (June 19th) stages, when spray damage was more likely to occur. The eighth treatment was lime-sulphur (1%) and lead-arsenate paste (0·4%) and ferrous sulphate. The wetter, but not the nicotine, was omitted from the Petal Fall spray.

* "Lethalate" Standard Wetting Preparation, at 0·05% w/v.

Records.

A severe May frost ruined the crop in 1935 ; records were therefore confined to the foliage. Heavy leaf-drop started in June after the second post-blossom application, and was complete early in July, when the fallen leaves were gathered up and counted. In addition, the remaining leaves on thirty "rosettes" (the whorls surrounding potential fruit buds on short, fruitless spurs), chosen at random on each tree, were counted.

There was a good crop in 1936, especially of Worcester, Cox being rather erratic. The dropped fruitlets were counted on three occasions for every tree of each variety, and the crop similarly at harvest. A count was made of rosette leaves as in 1935, but the numbers of dropped leaves were not recorded.

The results are summarized in Table I as means per tree (five per variety per treatment).

Spray damage.

The leaf records clearly show that the most phytotoxic treatments were those without ferrous sulphate ; in 1936, both varieties were significantly more damaged by lime-sulphur with calcium-arsenate powder (=approx. 40% As_2O_5) than by lime-sulphur either alone or with lead-arsenate paste (=approx. 16% As_2O_5), but in 1935 this was true only for Worcester Pearmain (leaf-drop). The inclusion of ferrous sulphate in the calcium-arsenate mixture restored the balance enough to show a significant interaction response on Worcester in 1936. When judged by counts of dropped leaves, all treatments damaged both varieties in 1935, chiefly as a result of the second post-blossom application. The criterion of rosette leaves remaining when leaf-drop had ended was clearly the less sensitive in spite of the uncertainty of counting dropped leaves, especially in windy weather. It is, however, not really feasible to collect and count all the leaves, though a sampling method might be practicable. Hessian troughs were erected around trees in former experiments (Frampton, 1928 ; Moore, 1930) to facilitate the task, but their construction and maintenance is tedious and their presence may influence the metabolism of the trees.

The zinc-sulphate mixture was more disastrous than the 1935 leaf-drop data suggest, for defoliation, preceded by leaf-roll, began soon after the Petal Fall application, and many of the dropped leaves had disappeared from these headland trees before recording began. This material was therefore not tested again. Defoliation was, in general, more severe in 1936, when the trees were carrying a crop, than in 1935 when they were not, and Cox suffered more severely than Worcester in both seasons. Up to the second post-blossom (Fruitlet) application, the only damage seen (apart from the above) was slight leaf-drop on Cox sprayed with lime-sulphur and either lead or calcium arsenate.

The fruit records leave no doubt that ferrous sulphate, while mitigating fruit-drop, failed to prevent its severity. On the Cox crop in 1936, as on the leaves of Worcester, it showed a significantly greater response with calcium than with lead arsenate. The 1936 test was severe, for the Fruitlet spray was applied in hot, sunny weather.

The weights of prunings were studied each year to see whether severe leaf-drop was reflected in the amount of young growth produced. The figures for Cox in 1936 are included in Table I, but the other data are omitted as they showed no

TABLE I.

Defoliation and Fruit Drop, 1935-36.

Spray.	Cox's Orange Pippin.						Worcester Pearmain.				
	Leaves.			Fruit.		Shoots.	Leaves.			Fruit.	
	No. dropped.	No. in rosette.		No. set ‡	% picked.	Weight of prunings.	No. dropped.	No. in rosette.		No. set ‡	% picked.
	1935.	1935.	1936.	1936.	1936.	1936.	1935.	1935.	1936.	1936.	1936
						lb.					
1. L-s. (1-60) ..	1,009	5·5	3·3	168	0·0	5·8	158	6·0	4·7	197	5·4
2. L-s.+nil+f.s.	478	5·7	5·8	127	11·8	5·3	119	5·8	5·3	219	6·7
3. L-s.+c.a. ..	1,003	4·9	2·4	165	0·0	4·7	419	5·3	3·0	280	2·6
4. L-s.+c.a.+f.s.	431	5·8	5·3	93	5·9	6·4	134	6·1	5·7	242	7·9
5. L-s.+l.a. ..	747	4·6	3·1	308	4·7	3·1	198	6·0	4·2	228	1·4
6. L-s.+l.a.+f.s.	458	5·8	5·2	234	2·1	4·2	115	6·0	5·5	184	4·5
7. Nil	**344**	**5·8**	**6·1**	**212**	**54·2**	**3·2**	**93**	**6·0**	**5·4**	**216**	**27·5**
8. (1935) L-s.+l.a.+z.s. (1936) L-s.(1%)+l.a.+f.s.	605	4·4	5·3	49	7·2	3·9	279	5·0	5·9	141	4·3
	STATISTICAL ANALYSIS.										
Effects of:					†	† ++	+				†
Spraying (i.e., 7 v. mean* of 1-6)	ss		sss		sss	ss	ss		ss		sss
Arsenate (a.) ..			s	ss		s	s		s		
Ferrous sulphate (f.s.)	sss	ss	sss		ss		sss		sss		s
Interaction (a. with f.s.) ..					s				sss		

‡=dropped fruitlets+picked fruits and windfalls.
+=correction made for surface cultivation which disturbed some of the leaves.
*=but not necessarily of any individual result.
s=significant ($0·01 < P \leq 0·05$).
ss=highly significant ($0·001 < P \leq 0·01$).
sss=very highly significant ($P < 0·001$).
++=correction made for stem girth, 1934.
†=these data were dealt with in transformation.

significant influence of treatments. Unsprayed control trees yielded less prunings by weight than any of the sprayed trees except those receiving lime-sulphur and lead arsenate. Loss of vigour on unsprayed trees can be attributed to uncontrolled pest and disease attacks, but it is nevertheless remarkable that these factors could in two years outweigh the severe damage done by some of the sprays. The reason for the poor vigour of trees sprayed with lime-sulphur and lead arsenate is not clear, for, though the least vigorous according to mean stem-girth, they were consistently less severely damaged than trees receiving lime-sulphur and calcium arsenate. The same two divergent treatments showed a nearly significant divergence also in 1935, and as the results do not seem reasonable, some unexplained factor is probably involved. The inclusion of ferrous sulphate in sprays containing arsenate led to increased weight of prunings, but this failed to reach significance although the iron-sprayed foliage was much darker and more luxuriant in both seasons, so that the trees required more spray-fluid.

It will be observed that increase in the dilution of lime-sulphur to 1-100, with lead arsenate and ferrous sulphate, did not effect any marked reduction in fruit-drop on either variety.

Other data.

As the primary object was to study spray damage, the incidence of pests and diseases received only secondary attention, more especially as the occurrence of severe leaf-drop would tend to interfere with their normal distribution. Certain records of Scab and the Red Spider were, however, made.

Apple Scab infection of foliage was assessed in July and September, 1935, by means of a category (0-5) for each tree ; too few fruits remained on damaged trees to provide reliable data. The disease was very slight indeed (average 0+) on all sprayed foliage on both occasions, showing that the inclusion of ferrous sulphate had not impaired fungicidal value ; but unsprayed foliage showed fairly severe infection (average for Cox: 4·4, 3·7; Worcester: 3·0, 2·6). The disease was practically non-existent except on controls in 1936, when *all* trees were routine sprayed twice pre-blossom before differential treatments began.

Red Spider infestation on foliage was similarly assessed by categories (0-5) allotted by general inspection in September, 1935. On sprayed trees of both varieties infestation was low (average 1·5), but the controls averaged 3·0 for Cox and 4·3 for Worcester. There was a marked increase on sprayed trees adjacent to controls. Infestation was high on the spare trees sprayed with the zinc-sulphate mixture, averaging 4·0 for each variety. Zinc sulphate, but not ferrous sulphate, had apparently nullified the acaricidal value of this lime-sulphur-arsenate mixture.

Drought caused further leaf-drop in the summer of 1935 on Cox, but not on Worcester. Trees sprayed with ferrous mixtures (treatments 2, 4, 6) then showed most loss, while those most damaged earlier by spray (treatments 3, 5) were not affected. Unsprayed trees and those sprayed with lime-sulphur only (treatment 1) were but moderately affected.

1937 Trial.

Ferrous sulphate had not solved the fruit-drop problem, so a further trial on the same trees in 1937 was based on the post-blossom use of more dilute lime-sulphur (1-100), as normally employed for Scab control. Seven treatments were

again compared in the same randomized arrangement, and as far as modifications allowed the trees were sprayed as before.

Sprays.

Only treatments 3 and 4 were changed, calcium arsenate being replaced by colloidal lead arsenate (0·4%) in treatment 3 and by lead-arsenate powder (0·2%≡0·4% paste≡0·4% colloidal) in treatment 4, ferrous sulphate being omitted here.

To afford a link with the previous trials, the spare trees were sprayed with lime-sulphur (1-60) plus lead-arsenate paste (0·4%) plus ferrous sulphate. Ferrous sulphate was used as before at 4 lb. crystals per gallon of lime-sulphur, irrespective of dilution.

All trees, controls included, were routine sprayed at Green Cluster (April 15th) and Pink Bud (April 30th) with lime-sulphur (1-100) plus lead-arsenate paste (0·4%), and with nicotine (0·05%) against Sawfly at Petal Fall. Sprays were applied at approximately 300 lb. pressure per sq. in. from a mobile 2 h.p. machine through a double nozzle, and averaged approx. 1 gal. per tree at Petal Fall (May 24th) and a little more at Fruitlet stage (June 14th).

Records.

The dropped fruits were counted on three occasions for every tree of each variety, and the crop similarly at harvest. Full notes but no counts were made of leaf-drop as it occurred. Certain data on Red Spider (see " Other Data ") were also obtained.

Spray damage.

The following notes were made during the season :

June 4th.—(11 days after Petal Fall spray). No apparent damage by any of the sprays.

June 26th.—(12 days after Fruitlet spray). No damage by any spray containing ferrous sulphate, not even with lime-sulphur at 1-60 on the spare trees ; " June drop " beginning, but no obvious differential fruit-drop ; slight scorch on oldest leaves of trees sprayed with l-s. alone (treatment 1) ; l-s. with each of the three arsenates causing moderate leaf-scorch all over the tree, and slight leaf-drop.

June 28th.—L-s.a. causing leaf- and fruit-drop freely on Cox ; practically no leaf-drop where ferrous sulphate used except when l-s. 1-60, then slight on Cox ; unsprayed control trees showing no damage ; trees sprayed with ferrous sulphate conspicuous by their dark green leaves.

July 8th.—Heavy fruit-drop on all Cox except unsprayed trees, moderate on Worcester ; moderate to severe leaf-drop on Cox with each of the arsenates, but only slight wherever ferrous sulphate used and on trees sprayed with l-s. alone ; practically no leaf-drop on any Worcester tree. Evidently Worcester is much more resistant to spray damage than Cox ; ferrous sulphate, while obviating leaf-drop, has again failed substantially to check fruit-drop.

August 18th.—General confirmation of position on July 8th, but drought symptoms have supervened and Cox, but not Worcester, has lost many more leaves. Only the control trees among Cox now show little leaf-drop ; those sprayed with lime-sulphur only are intermediate. A category figure for leaf-drop in the range 0-3 was allotted to each Cox tree ; the means per treatment, together with those for crop, are set out in Table II.

TABLE II.

Defoliation, Fruit Drop, and Red Spider, 1937.

Post-blossom spray.	Cox's Orange Pippin. Leaf-drop in August.	Cox's Orange Pippin. No. fruit set.*	Cox's Orange Pippin. % picked.	Worcester Pearmain. No. fruit set.*	Worcester Pearmain. % picked.	Worcester Pearmain. Red Spider eggs on: Fruit. Max. 3·0	Worcester Pearmain. Red Spider eggs on: Branches. Max. 5·0
1. L-s. (1-100)	1·4	201	6·1	203	18·0	1·2	2·2
2. L-s.+nil+f.s.	2·0	188	14·0	263	24·9	2·4	4·0
3. L-s.+l.a. (coll.) ..	1·8	157	3·9	156	27·1	1·0	2·2
4. L.s.+l.a. (powder) ..	2·4	182	1·9	204	18·8	1·5	2·5
5. L-s.+l.a. (paste) ..	2·0	279	6·7	274	22·2	1·6	2·4
6. L-s.+l.a. (paste)+f.s...	1·8	185	9·8	213	27·5	2·0	4·3
7. Nil	**0·6**	**182**	**55·6**	**240**	**35·8**	**1·6**	**2·2**
8. L-s. (1-60)+l.a. (paste) +f.s.	2·7	129	4·0	188	23·5	1·8	3·0

STATISTICAL ANALYSIS.

	Cox.	Worcester.
Percentage fruit picked:	No. 4, 3, 1, 5, 6, 2, 7 ..	Not significant.
Red Spider (summer eggs)	.. ..	No. 3, 1, 4, 5, 7, 6, 2.
Red Spider (winter eggs)	.. ..	No. 3, 1, 7, 5, 4, 2, 6.

* Dropped fruitlets+picked fruits and windfalls.

The data confirm that ferrous sulphate (treatments 2 and 6) failed to solve the fruit-drop problem even with lime-sulphur at 1-100, but it showed slight mitigation on Cox. As regards leaf-drop, the only significant result at this late stage was the one between unsprayed trees and the mean of the rest; the beneficial effect of ferrous sulphate seen earlier in the season was no longer evident after the onset of drought leaf-drop. The spare trees (treatment 8) were more severely affected than the others. Lead arsenate was apparently most harmful in powder form, but the difference was not quite significant.

OTHER DATA.

Apple Scab infection was very slight, and examination of the data obtained was unprofitable. These trees had always proved easy to protect from Scab, and pre-blossom sprays, applied to all trees including controls, were evidently enough.

Red Spider. When the Worcester crop was being graded, summer eggs were observed in the "eyes" of the fruit, and as differences were apparent, a rough estimation was made by allotting category figures—1 (slight or none) to 3 (very obvious)—for each tree. The means are shown in Table II; spray treatment did not lower the quantity of eggs deposited, and most Spider occurred on trees

receiving ferrous sulphate. Later, an independent observer* made a similar category estimation (0-5) of winter eggs on shoots and branches of the same trees with the same result.

DISCUSSION.

During three seasons of variable character (Chapelow and Tydeman, 1936-38), though on young trees on a single plot, it has consistently been shown that the addition of ferrous sulphate to lime-sulphur sprays (to precipitate the mono-sulphide prior to spraying) prevented much early leaf-drop and some fruit-drop; it failed, however, to arrest fruit-drop sufficiently to justify its use commercially on Cox. This suggests that fruit-drop, at least, is not caused exclusively by the mono-sulphide as such. The retention of foliage in early summer by the use of ferrous sulphate was not proof against loss of much of it later. The onset of what appear to be drought symptoms may in fact be partly a delayed effect of earlier spraying, as mentioned by Kelsall, *et al.* (*loc. cit.*) in regard to the aluminium-sulphate mixture; for unsprayed trees, though like the ferrous-sprayed ones hitherto carrying practically full foliage, were but little affected. It may be that ferrous sulphate merely wards off the inevitable for a time, and that ultimate damage is a function of the residual sulphur and the green plant in contact with it.

There was no evidence that lime-sulphur, either alone or with arsenate, suffered in fungicidal efficiency from the inclusion of ferrous sulphate, which, however, depressed the acaricidal value of lime-sulphur at 1-100, but not at 1-60. The ferrous sprays, being black and persistent, were objectionable for the workers, though these properties show up faulty spraying and ensure good weathering of the deposit. Ferrous mixtures produced a strong odour of hydrogen sulphide in the spray-tank; Kelsall, *et al.* reported otherwise.

Calcium-arsenate powder was throughout more phytotoxic than lead-arsenate paste of lower As_2O_5 equivalent, but the inclusion of ferrous sulphate in the mixtures restored the balance. At dilutions giving equal As_2O_5 equivalent their phytotoxicity would probably be more nearly similar. Kelsall *et al.* found it desirable to reduce the strength of calcium arsenate for post-blossom application. Lime-sulphur (1-60) post-blossom was equally phytotoxic with or without lead arsenate. When used alone at 1-100 it gave only slight early leaf-drop on Cox, yet its lead-arsenate mixture still caused severe early leaf-drop, due mainly to arsenical derivatives of the reaction, but obviated by ferrous sulphate. Similar arsenical damage (Moore, 1946) occurred on Cox on a nearby plot in the same year (1937), when *weak* lime-sulphur with lead arsenate was held responsible. Fruit-drop was severe from lime-sulphur at either dilution (except at 1-100 on Worcester) alone or with arsenate, and was but little mitigated by ferrous sulphate. Lead arsenate in powder form proved rather more phytotoxic than colloidal or paste forms, but can be more readily handled.

Zinc sulphate, used in 1935 as an alternative to ferrous sulphate, proved highly phytotoxic, and further trial of this material was therefore abandoned. The evidence suggested that it nullified the acaricidal value of lime-sulphur with lead arsenate. Kelsall *et al.* stated that "Typical lime-sulphur injury was largely eliminated by the use of zinc sulphate". This does not accord with the above,

* The writer is indebted to Mr. W. Steer for these observations.

which, however, refers to the lead-arsenate mixture. There is, nevertheless, sufficient evidence herewith of differences in the phytotoxic responses of the susceptible Cox and the much more resistant Worcester growing alongside each other to account for any apparent aberrations in comparison with Canadian varieties under Canadian conditions, apart from any effects of slight differences in dilution of the spray materials tested.

It is worthy of emphasis that the second post-blossom application was responsible every year for nearly all the damage, and that spray-induced fruit-drop was delayed until the end of June, when it was revealed as excessive "June-drop". There appears to be some functional relationship between these two phenomena (Moore, 1946). Neither variety could retain its crop following a mid-June spray containing lime-sulphur at 1-60, though Worcester was on the whole the less devastated. Accumulated data and experience over many seasons emphasize that in many parts of S.E. England cropping Cox's Orange Pippin and several other varieties do not tolerate even 1-100 lime-sulphur applied alone in June (and see Grubb, 1924), and as ferrous sulphate has not solved the fruit-drop problem, lime-sulphur must either be used even more dilute and without arsenate or be avoided there at that period.

SUMMARY.

The addition of ferrous sulphate to lime-sulphur at 1-60 applied post-blossom with or without lead or calcium arsenate greatly reduced early spray-induced leaf-drop present on apple varieties Cox's Orange Pippin and Worcester Pearmain, but failed to prevent, though mitigating, severe fruit-drop on both varieties. There was no evidence that the addition impaired the fungicidal value of the combined spray. Calcium-arsenate powder (0·4%) proved more phytotoxic than lead-arsenate paste (0·4%) of much lower As_2O_5 equivalent. With lime-sulphur at 1-100, the paste and a colloidal form (0·4%) were each rather less phytotoxic on Cox than a powder form (0·2%) of double As_2O_5 equivalent. The addition of ferrous sulphate to the combined spray prevented early leaf-drop on Cox, but failed substantially to prevent fruit-drop, and also depressed acaricidal value. Zinc sulphate was an unsatisfactory alternative to ferrous sulphate. Worcester proved much less susceptible than Cox to spray damage, most of which was caused by the second post-blossom (June) application.

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SPACING AS A FACTOR GOVERNING ROOTING AND GROWTH OF HARDWOOD CUTTINGS OF THE MYROBALAN B PLUM ROOTSTOCK

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Introduction.

Studies on spacing have already been carried out in connection with various types of crop ; the present paper deals with the factor of spacing in relation to the propagation of fruit tree rootstocks from hardwood cuttings. This method of propagation has only a limited application in the raising of rootstocks, notably with Myrobalan B plum (*Prunus cerasifera* Ehrh.), the variety used in the present experiments. The ultimate aim, however, will be to extend the use of hardwood cuttings to a wider range of stocks, and so to supplement, or even replace in some cases, the methods of stooling and layering.

EXPERIMENTAL PROCEDURE.

Three main experiments were carried out. In the first, in 1942-43, the effects of varying the distance between the rows of cuttings—the distance between the cuttings themselves in the rows remaining constant—was investigated. It was on two dissimilar soils, (1) a typical loam, and (2) a specialized lake soil, highly calcareous and fine textured, with an admixture of peat and a definite peat layer at a depth of 15 to 18 inches. The behaviour of fruit-nursery and agricultural indicator crops on this soil has already been described (Garner and Roach, 1945). The second experiment, in 1944-45, was a repetition of the first with slight modifications, and was conducted on the same two soils. In the third experiment, in 1944-45, the effect of irrigation on the spacing relationships of the plants derived from cuttings, on the loam only, was studied. A " square-plant " was used, with the distance between individual cuttings varying in two directions at right angles to one another.

In all three experiments the cuttings were obtained from the same source, namely, a mature hedge known to be a reliable, indeed the best available, source of such cuttings (Sinha and Vyvyan, 1943). The cuttings were taken early in November, within the optimal period for the Myrobalan B rootstock (Vyvyan, 1943, Hatcher and Garner, unpublished). They were 12 inches in length, and were planted vertically in such a way that only the top two or three inches projected above ground level, as described by Garner (1944).

INTER-ROW SPACING EXPERIMENTS ON TWO SOILS.

Experiment I. 1942-43.

Description of experiment. Four spacings between the rows were adopted, viz. 4½, 9, 18 and 27 inches, the last being the usual distance employed for horse-cultivation. Along the rows spacing was constant at 2 inches. Unit plots were of

equal area, 2 ft. × 9 ft., with decreasing numbers of rows, viz. 25, 13, 7 and 5, respectively, with increasing spacing between them. The individual rows were 2 ft. in length and contained 10 experimental cuttings, with guard cuttings at their ends. The unit plots were arranged in two parallel linear sets of 8, so as to give 4 randomized blocks. The design was duplicated on the two soils, and there were 32 unit plots altogether, with 31 degrees of freedom.

The cuttings were made and planted on November 12th and 13th, 1942, altogether 4,800 cuttings being used. After a year's growth a record was made of each cutting, and wood growth measurements were made in the field. From these records information based on the total crop was available, though, in summarizing, only the 10 experimental cuttings of each row were included. Further, the outside rows of the unit plots with 4½ in. and 9 in. spacings were excluded, a procedure unnecessary for the wider spacings of 18 in. and 27 in., in which edge effects were not expected to be large. In addition, random samples, of 10 cuttings from each plot, were carefully dug for further observations on: (*a*) the entire plant (including photographic record), (*b*) more detailed wood growth measurements, and (*c*) weight determination after dismemberment into primary shoots, secondary shoots, carcase, basal roots and side roots. In this way quantitative data concerning the growth of the cuttings at the different spacings, and in the two soils, were secured.

Results.

(i) *Establishment of cuttings.*—In the first section of Table I the percentage of established cuttings is given for all spacings, together with the results of analysis of variance. This analysis shows that while there was a significantly higher establishment on the loam (72·9 per cent. as against 64·1 per cent. on the lake soil, $P<0\cdot01$) there was no effect of spacing on establishment in either soil. This result is important for two reasons; first, any effects of row spacing have not been caused by a differential establishment rate, and second, should any disadvantage be gained by close spacing of the rows, this is not offset by a lower percentage stand.

(ii) *General growth of cuttings.*—There was a clear cut, visible, effect of both soil and spacing on total growth, the one-year plants on the lake soil being considerably larger than those on the loam, with minimal size on each soil at the closest spacing. This is seen clearly in the photographs of representative groups of cuttings reproduced in Plate I. Weight determinations made on the sample cuttings are presented graphically in text Fig. 1. The uppermost curve represents total weight, and the space below the curve is divided into regions proportional to the weights of the various parts into which the plants were cut. It is evident that: (i) the plants on the lake soil were bigger than those on the loam soil, (ii) there was an increase in size with increased spacing up to 18 in., and (iii) there was no increase in size, but a tendency to diminish, as a result of increasing the spacing from 18 in. to 27 in. Statistical analysis confirmed these results and, despite the very different degrees of growth on the two soils, similar spacing effects were obtained, the soil-spacing interaction variance being insignificant.

(iii) *Distribution of growth: Fresh weight data.*—Table II summarizes the fresh weight records and, in addition, gives ratios obtained by comparing different parts of the plants. As text Fig. 1 demonstrates, the variation among the different parts due to soil and spacing resembles that shown by the whole plant, which represents a summation of these partial effects; nevertheless the relationship between the parts

TABLE I.

Percentage establishment of cuttings for all experiments.

I. *Inter-row spacing experiment, 1942-43.*

Loam.				Lake Soil.				Analysis of variance. F Values.		
4½ in.	9 in.	18 in.	27 in.	4½ in.	9 in.	18 in.	27 in.	Soil.	Spacing.	Soil × Spacing. Interaction.
74·3	73·5	69·3	74·5	62·3	68·0	60·8	65·5	16·72**	1·43NS	0·62NS

II. *Inter-row spacing experiment, 1944-45.*

Loam.			Lake Soil.					
6 in.	15 in.	27 in.	6 in.	15 in.	27 in.	Soil.	Spacing.	Soil × Spacing. Interaction.
58·4	69·2	49·0	74·2	66·6	78·8	22·15***	0·49NS	9·40**

III. *Irrigation spacing experiment, 1944-45.*

3 in.				9 in.							
Not irrigated.		Irrigated.		Not irrigated.		Irrigated.					
Not manured.	Manured.	Not manured.	Manured.	Not manured.	Manured.	Not manured.	Manured.	Manure.	Irrigation.	Spacing.	Inter-actions.
60·0	74·7	78·7	81·3	56·0	42·7	56·0	53·3	<1·00NS	16·60*	25·44***	NS

NS Not significant.
* Significant P <0·05.
** ,, P <0·01.
*** ,, P <0·001.

is also influenced, as becomes evident from an examination of their ratios, three of which have been selected for statistical analysis :—

(*a*) Ratio of shoot to root.—Rogers and Vyvyan (1934) have shown that on a sandy soil the amount of stem growth to a given weight of root is rather less than half that on a loam, and that, on a clay soil, an even greater proportion of stem is formed. These authors used trees up to 10 years of age and expressed their ratio as stem to root, the stem including the trunk. In 1-year-old plants from cuttings, in which the original carcase has produced shoots from the apex and roots from the base, it is more interesting to compare the relative amounts of this new growth, disregarding

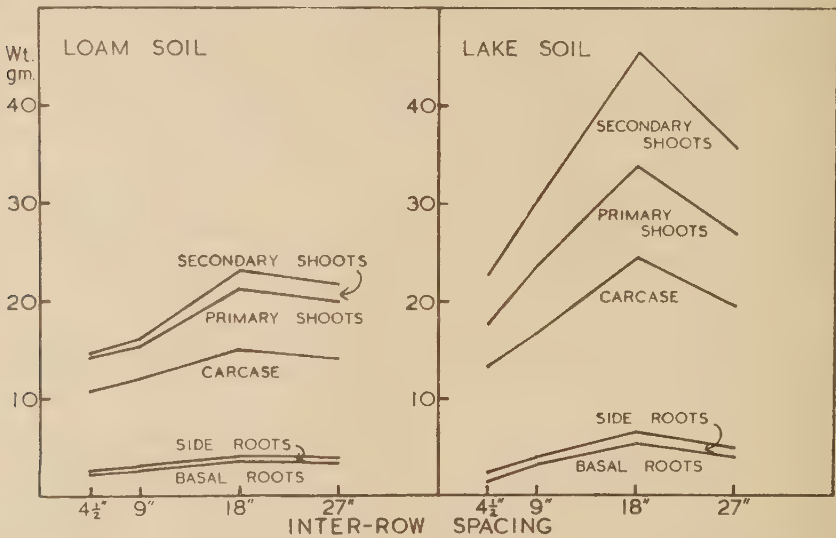

FIG. 1.

Inter-row spacing experiment, 1942-43, on two soils. Graphs showing the effects of spacing and soil on total weight of the cutting after one year's growth, and on the distribution of that weight in various regions of the plant.

the growth of the carcase. Comparing the shoot : root ratios of the two soils (Table II, item 5), it will be observed that much higher values were reached by the plants grown in the lake bed than in the loam, this being in accordance with the finding of Rogers and Vyvyan. As regards spacing, on the loam the more widely spaced plants gave the higher shoot : root ratios, whereas on the lake soil the reverse was the case. The reason for this cannot be stated with certainty, but it should be noted that at the closest spacing (4½ in.) the amount of root per cutting was approximately equal on the two soils, while at the wider spacings the lake plants produced considerably more root (Table II, item 3). This shows that in spite of the strong root competition on both soils at the closest spacing, the growth of the aerial portion of the plant (as distinct from the roots) is determined by the type of soil, and that the "efficiency" of the roots of the lake-soil plants is higher. This higher efficiency of the roots is seen at all spacings, though the shoot : root ratio shows that on the lake soil the relative amount of shoot made per unit of root decreases with increased spacing, whereas on the loam soil it increases.

TABLE II.

Inter-row spacing experiment, 1942-43. Fresh weight records of sample cuttings (gm.).

	Loam.				Lake soil.				Analysis of variance. F. values.		
	4½ in.	9 in.	18 in.	27 in.	4½ in.	9 in.	18 in.	27 in.	Soil.	Spacing.	Soil × Spacing, Interaction.
1. Carcase	8·22	9·11	10·99	10·57	10·97	12·92	18·03	14·82			
2. Shoots	3·92	4·19	8·05	7·48	9·39	13·58	21·00	15·98			
3. Roots	2·42	2·81	3·99	3·72	2·31	3·86	6·37	4·85			
4. Total weight	14·56	16·11	23·03	21·77	22·67	30·36	45·40	35·65	42·68***	9·25**	0·60
5. Shoot:root ratio	1·58	1·50	1·97	2·04	3·89	3·52	3·16	3·05	124·59***	0·70NS	4·96*
6. Side roots	0·18	0·15	0·35	0·22	0·39	0·77	1·13	0·90			
7. Basal roots	2·24	2·66	3·64	3·50	1·92	3·09	5·24	3·95			
8. Side root:basal root ratio	0·082	0·057	0·092	0·051	0·198	0·271	0·241	0·201	89·09***	1·09NS	1·42NS
9. Secondary shoots	0·38	0·77	1·90	1·74	5·06	6·73	11·58	8·52			
10. Primary shoots	3·54	3·42	6·15	5·74	4·33	6·85	9·42	7·46	60·68***	6·61**	1·06NS
11. Secondary shoot:primary shoot ratio	0·113	0·222	0·294	0·306	1·215	1·030	1·230	1·090	216·49***	4·19*	4·52*

NS Not significant.
* Significant P <0·05.
** ,, P <0·01.
*** ,, P <0·001.

(*b*) Ratio of side roots to basal roots.—The variations in the root systems characteristic of the two types of soil can be assessed from the data given in Table II on side and basal roots, respectively (items 6 and 7). The former are produced from the sides of the cutting, the latter from the basal callus. On the lake soil a larger proportion of the total, as well as a larger absolute amount of side root was produced, and one may conclude that the lake soil favours both types of roots but, as the ratios indicate, preponderantly the side roots. Thus soil effect is highly significant ($P < 0 \cdot 001$), but spacing and its interaction with soil are insignificant.

(*c*) Ratio of secondary shoots to primary shoots.—Those shoots which arise directly from the cutting are regarded here as primary, while those which arise from buds on the primary shoots are classed as secondary.

Primary shoot growth (Table II, item 10) is greater on the lake soil than on the loam at all spacings, this being a highly significant effect ($P < 0 \cdot 001$). Secondary shoot production (Table II, item 9) on the lake soil likewise greatly exceeds that on the loam ; but the distribution of the shoot system as between the two types is also affected significantly by the soil factor, as is shown by their ratio (Table II, item 11). This ratio for the loam varies from 0·11 to 0·31 over the range of spacing, whereas on the lake soil it is always greater than unity (1·03 to 1·23). As the difference in response to spacing on the two soils is sufficiently great for the interaction soil and spacing to reach a significant level ($P < 0 \cdot 05$), the distribution of growth between the primary and secondary shoots in fact resembles that already seen in the relation of side to basal roots, the lake soil in both cases favouring the branching habit. These effects are clearly seen in the photographs reproduced in Plate I.

(iv) *Shoot growth relationship*.—The data assembled in Table III show the following relations :—

(*a*) The number of primary shoots varies between two and three per cutting and is almost independent of soil and spacing. The differences already noted in the weight data are therefore attributable to variable response in growth of the shoots. The total length of primary shoots increases with wider spacing on both soils, but the greater total length at comparable spacing is always found on the loam. The same holds good for the average length of individual primaries.

(*b*) As regards secondary shoots, striking differences in both shoot number and shoot length are found on the two soils. Both characters increase with wider spacing and are very much greater on the lake soil than on the loam, to which the very large effects on the weights already noted can be related. Even taking account of the varying number of secondary shoots formed on the two soils, the mean length of the secondaries on the lake soil is still nearly double that on the loam. Spacing effect on individual secondary shoot length is absent on both soils, so that the effect on total length is due entirely to the number of such shoots at the different spacings.

(*c*) So far as the effects of spacing on the comparative growth and number of the two categories of shoots are concerned, the results are identical with those discussed in connection with the ratios of their fresh weights in Table II.

(*d*) The effect on branching of the primaries is clearly brought out in item 9 of Table III, in which the percentages of unbranched primaries are given. Soil effects are very significant ($P < 0 \cdot 001$), with a much greater branching on the lake soil. Spacing has little effect on the lake soil in this respect, but branching on the loam increases with wider spacing, and the F value of 3·18 for spacing is significant.

TABLE III.

Inter-row spacing experiment, 1942-43. Shoot growth records of sample cuttings.

	Loam.				Lake soil.				Analysis of variance. F values.		
	4½ in.	9 in.	18 in.	27 in.	4½ in.	9 in.	18 in.	27 in.	Soil.	Spacing.	Soil × Spacing. Interaction.
Number of shoots.											
1. Secondary	1·65	3·60	7·65	8·15	8·40	9·28	13·45	12·05			
2. Primary	2·33	2·78	3·08	2·98	1·08	2·53	2·65	2·75			
3. Secondary : primary ratio ..	0·71	1·34	2·47	2·78	4·15	3·68	5·04	4·75	69·50***	9·96***	5·62***
Total length of shoots (cm.).											
4. Secondary	11·4	23·4	55·8	61·1	121·0	145·4	212·7	177·7			
5. Primary	49·2	50·4	62·4	61·5	29·7	44·3	47·1	45·7			
6. Secondary : primary ratio ..	0·23	0·46	0·86	0·99	4·20	3·38	4·45	4·39	274·57***	9·26***	7·39**
Mean length of shoots (cm.).											
7. Secondary	8·0	6·4	7·1	7·6	14·4	15·7	15·4	14·1			
8. Primary	21·2	18·2	20·3	21·1	14·6	17·8	17·7	16·6			
9. Percentage of unbranched primaries	66·7	63·2	49·7	44·5	27·2	32·7	25·2	27·5	63·43***	3·18*	1·85NS

NS Not significant.
* Significant P <0·05.
** ,, P <0·01.
*** ,, P <0·001.

EXPERIMENT II. 1944-45.

Description of experiment.—This experiment was a repetition, with slight modification, of the previous one, to test three inter-row spacings which could be adopted for different cultural methods, 27 in. being suitable for horse cultivation and 15 in. for hand cultivation between the rows, whilst 6 in. necessitates cultivation from the ends of the rows arranged in beds. The distance apart of the cuttings within the row was always 2 inches, as in the first experiment, and the rows of 30 cuttings each were 5 ft. in length. Unit plots were of approximately equal area and contained varying numbers of rows according to spacing, viz. 11 for 6 in., 5 for 15 in., and 2 for 27 in. spacing. With the 6 in. and 15 in. spacings, 2 rows in each case were selected from which sample cuttings were taken. The unit plots were arranged in randomized blocks of three with each spacing represented once, and there were 5 blocks. This arrangement was duplicated on the two soils, giving 30 unit plots altogether, with 29 degrees of freedom. The loam plots were planted on November 11th, 1944, and the lake plots 5 days later ; 5,400 cuttings were used in addition to guard row cuttings. After a year's growth records were made of all cuttings to determine degree of establishment, and from the selected rows in each unit plot, 8 sample cuttings were harvested for wood growth records. Owing to heavy pressure of other experiments, it was decided not to dig the plants for weight determinations.

RESULTS.

(i) *Establishment of cuttings.*—The percentage of established cuttings is given for all treatments in the second section of Table I. The effect of soil is again highly significant ($P < 0 \cdot 001$), but in this experiment is in favour of the lake soil, whereas in the previous one the loam gave the better result. The effect of spacing on establishment is, on the average, not significant, though the greater variability on the loam as compared with the lake soil is sufficiently marked for the interaction of soil and spacing for each a highly significant value ($P < 0 \cdot 01$).

(ii) *General growth of cuttings.*—The general relations of growth to spacing in the two experiments described are brought together in text Fig. 2. It should be stated that the values for spacing shown in the diagram have been adjusted for differences of establishment and thus represent the mean area available per cutting. The contrasting behaviour of lake and loam soils clearly appears, the general level of growth being higher on the lake soil. The effect of spacing differs markedly in the two seasons, the salient fact being that with the closest spacing the growth made is the same in both seasons, though the effect of soil is most apparent with crowded plants. As spacing increased, very different responses were obtained in the two seasons. Thus, in 1942-43, the curves for lake and loam soils are, in general, parallel with an increasing spacing effect up to 50 sq. cm., which marks an optimum. In 1944-45, however, growth on the loam continued to increase with increased spacing up to the maximum used ; by contrast, on the lake soil, a comparatively small spacing effect appears so that the growth on the loam at the wide spacing surpasses that on the lake soil.

(iii) *Shoot growth relationships.*—The principal data are assembled in Table IV, and the following results are seen :—

(*a*) The number of primary shoots, as in the previous experiment, remains

Table IV.

Inter-row spacing experiment, 1944-45. Shoot growth records of sample cuttings.

	Loam			Lake soil.			Analysis of variance. F values.		
	6 in.	15 in.	27 in.	6 in.	15 in.	27 in.	Soil.	Spacing.	Soil × Spacing. Interaction.
Number of shoots.									
1. Secondary	2·13	4·80	5·20	5·87	6·17	7·54			
2. Primary	2·08	2·17	2·80	2·40	2·05	2·37			
3. Secondary : primary ratio	1·04	2·23	1·87	2·47	3·08	3·03	20·05***	5·52*	1·92NS
Total length of shoots (cm.).									
4. Secondary	27·5	90·4	132·0	78·6	86·0	109·7			
5. Primary	52·1	101·5	147·6	84·4	88·5	84·1			
6. Secondary : primary ratio	0·58	0·90	0·99	0·96	0·95	1·28	9·49**	5·61*	8·20**
Mean length of shoots (cm.).									
7. Secondary	12·5	19·4	24·9	13·5	13·1	14·3			
8. Primary	24·6	46·5	52·8	35·2	44·1	35·9			
9. Percentage of unbranched primaries	53·2	27·6	38·6	36·0	27·8	23·0	3·87NS	3·53NS	1·00NS

NS Not significant.
* Significant P <0·05.
** „ P <0·01.
*** „ P <0·001.

nearly constant at between two and three per cutting, being almost unaffected by soil type or spacing. The total length of primary shoots, on the other hand, shows a very large effect of spacing on the loam, and none on the lake soil; naturally, the same effect appears in the average length of primaries.

(*b*) In general, the effects of the factors on the growth of secondaries resemble those previously described. In this experiment, however, the range of total length

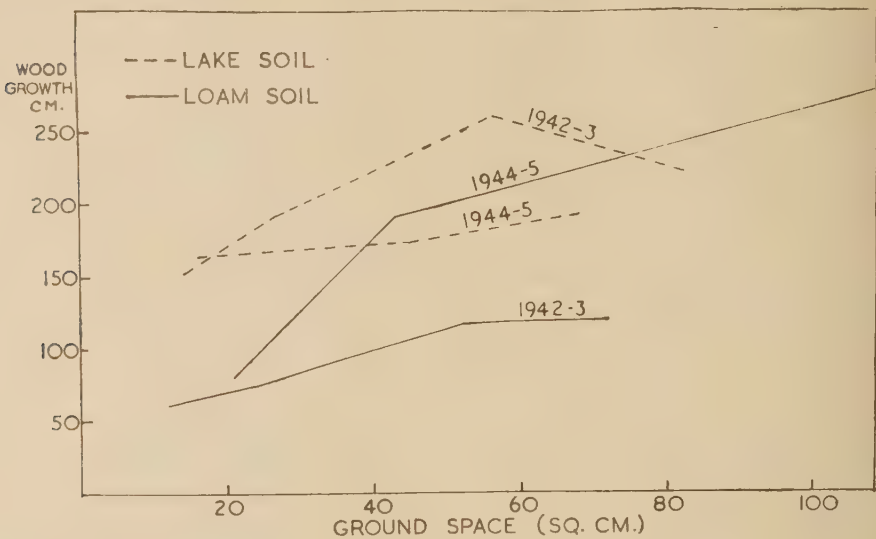

FIG. 2.

Inter-row spacing experiments. Graphical presentation of the effects of spacing, soil and season on shoot growth made by the cutting during its first year.

on the loam is greater than that on the lake soil, so that the response to spacing is much greater in the former. The same is true of the average length of secondaries, which is unaffected by spacing on the lake soil, and on the loam shows a wide range of values with spacing.

(*c*) The ratios of both number and total length of secondary to primary shoots display very highly significant variation, due to the soil factor, in both cases the higher value being associated with the lake soil. The main spacing effect in both cases is significant at the level $P < 0{\cdot}05$; moreover, the differential effect of spacing on the two soils on the ratio of total length of shoots is sufficiently great as to give an F value of 8·20 for the soil:spacing interaction, with $P < 0{\cdot}01$. As to branching, item 3 in Table IV indicates that the tendency to branch is much less on the loam than on the lake soil and that spacing effects are greater on the former.

(*d*) The same tendency is expressed in the data given in item 9 of this Table, in which the percentage of unbranched primaries is entered. These effects, however, in the season 1944-45, are much less than in 1942-43, as the F values entered in Tables III and IV clearly demonstrate.

IRRIGATION AND SPACING EXPERIMENT (LOAM SOIL)

Description of experiment.

The 1942-43 experiment had demonstrated contrasting effects on growth and branching of the two soils, and the aim of the third experiment, in 1944-45, was to ascertain if the lake soil effect could be reproduced on a loam by irrigation. The available results of the single experiment indicated that the greater degree of branching on the lake soil might be in some way related to the greater vigour of growth of the plants and their high shoot: root ratios. The hypothesis tested was whether the property of the lake soil responsible for the high degree of branching was associated with high water content, and the method adopted was to maintain artificially a high water content in the loam, and so induce the lake soil type of growth if possible.

The square plant used necessitated a novel design. Two planting distances were chosen, 3 in. square and 9 in. square, with 25 experimental plants per unit plot, also arranged as a square. With the wider spacing it was sufficient to enclose each square plot with a double row of guard cuttings; to achieve the closer spacing additional cuttings were planted at intermediate positions to give a 3 in. plant throughout the square. Unit plots were planted in pairs, one at each spacing, the order of the pair being at random. These double plots were arranged in six groups of two, with more guard rows buffering between them. This gave six blocks, one double plot of each block being irrigated, the other not, again in random order. Altogether there were thus 12 double plots, half of which were irrigated.

This was how the experiment was originally planned, but during the summer a manurial treatment was superimposed on half the plots, which reduced the number of blocks from 6 to 3, and increased the number of treatments to 8 with the following factorial combinations; manured and non-manured, irrigated and non-irrigated, close spacing and wide spacing. The method of statistical analysis was in consequence rather more complex than in the earlier experiments, and involved split-plot procedure.

The cuttings were planted on November 3rd, 1944, and harvested after one year's growth. The experimental cuttings were carefully dug and dismembered, and dry weight determinations were made of primary and secondary shoots, carcase, and basal and side roots.

The irrigation treatment was carried out by a hand watering can from a tank placed by the experimental plot. A check was kept on the soil moisture by the insertion of soil-moisture tensiometers (Rogers, 1936) in irrigated and non-irrigated plots at each of the two spacings, the porous pots being buried 8 in. deep, approximately level with the bases of the cuttings. The method was to irrigate the appropriate plots at a rate equivalent to 1 in. of rainfall immediately the soil-moisture tension in the driest plot rose to 25 cm. Hg.

Relative light intensities were measured on all plots at intervals throughout the growth period by the method fully described by Blackman and Rutter (1946).

Results.

(i) *Soil moisture.*—The moisture-meter records are presented graphically in text Fig. 3. The soil-moisture tension recorded is inversely proportional to the water content. Early in the season, up to the middle of June, soil moisture was adequate

in all the plots; but with the acceleration of growth, the demand for water by the plants sharply increased and the tension in the non-irrigated plots rose accordingly. The effectiveness of the irrigation in maintaining the soil moisture of the plots is quite evident from the graphs, but the much greater demand for water by the closely-spaced plots, and the correspondingly greater difficulty in maintaining its supply, should be noted. There is little doubt that the irrigation was quite adequate in providing plots of contrasting degrees of moisture content.

(ii) *Nitrogen application.*—During July it was seen that the foliage of the cuttings on the irrigated plots was much lighter in colour than that of those on the

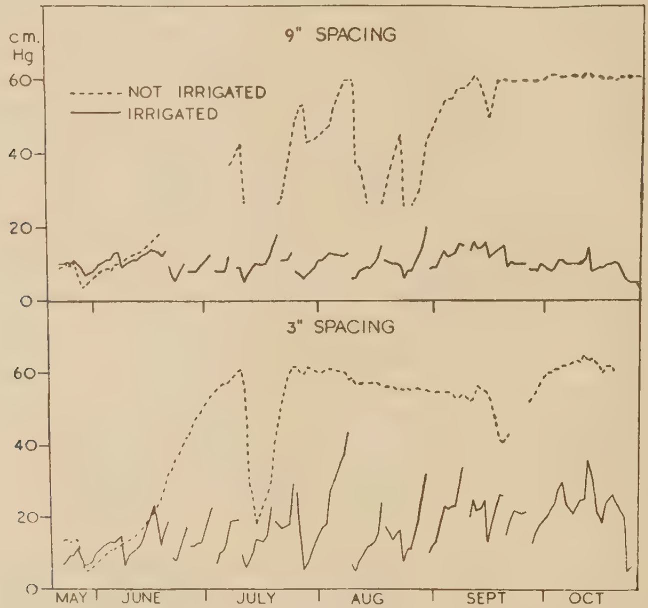

FIG. 3.
Irrigation and spacing experiment (loam soil), 1944-45. Soil moisture records taken on irrigated and non-irrigated plots at wide and close spacing throughout the growing season.

non-irrigated ones. As this became more distinct it was decided to apply dressings of potassium nitrate to half the plots in the experiment. The rate and time of application approximated to 1 cwt. KNO_3 per acre given immediately before irrigation on July 24th, August 18th and September 8th. Ten days after the first application the manured plots were seen to be slightly greener than the others, and they remained distinctly darker in colour to the end of the experiment. The cuttings in the manured plots also grew more vigorously, but the only evidence for greater leaf development was that derived from estimations of light intensity taken among the plants at random positions on all the plots. The data for light intensity are graphically shown in text Fig. 4, the ordinate representing the percentage of incident light absorbed by the foliage. The effect of treatments on leaf development clearly appear in the figure.

TABLE V.

Irrigation spacing experiment, 1944-45. Dry weight records of sample cuttings (gm.).

	3 in. spacing.				9 in. spacing.				Analysis of variance. F values.			
	Not irrigated.		Irrigated.		Not irrigated.		Irrigated.					
	Not manured.	Manured.	Not manured.	Manured.	Not manured.	Manured.	Not manured.	Manured.	Manure.	Irrigation.	Spacing.	Interactions.
1. Carcase	4·65	5·03	4·85	4·97	13·46	10·48	11·55	14·16				
2. Shoots	2·52	2·88	2·63	4·37	20·09	13·87	15·70	21·93				
3. Roots	1·14	1·24	0·93	1·20	5·42	4·26	4·15	5·07				
4. Total weight ..	8·31	9·16	8·41	10·54	38·97	28·60	31·39	41·15	<1·00NS	<1·00NS	212·88***	NS
5. Shoot:root ratio ..	2·20	2·29	2·74	3·38	3·72	3·28	3·75	4·27	2·59NS	11·73*	36·76***	NS
6. Side roots ..	0·13	0·13	0·05	0·10	0·86	0·59	0·24	0·42				
7. Basal roots ..	1·01	1·11	0·88	1·10	4·56	3·67	3·91	4·65				
8. Side root: basal root ratio	0·125	0·108	0·057	0·070	0·201	0·169	0·062	0·087	<1·00NS	7·29NS	1·47NS	NS
9. Secondary shoots	0·30	0·46	0·19	0·28	3·78	3·16	2·70	4·68				
10. Primary shoots ..	2·22	2·42	2·44	4·09	16·31	10·71	13·00	17·25				
11. Secondary shoot: primary shoot ratio	0·138	0·182	0·092	0·074	0·229	0·306	0·202	0·295	3·85NS	7·45NS	20·12***	NS

NS Not significant.
* Significant $P < 0{\cdot}05$.
** ,, $P < 0{\cdot}01$.
*** ,, $P < 0{\cdot}001$.

During May the effect of spacing becomes obvious, and the denser shade cast by the irrigated plants is quite prominent with the close spacing, though at wide spacing much less effect of irrigation is seen. The effect of manuring is relatively small and not consistent. There can be little doubt that improved water supply with close spacing has considerably increased foliage development, but whether by growth of individual leaves or by rate of leaf production due to branching, remains undetermined. With wide spacing there is no conclusive evidence of an irrigation effect.

(iii) *Establishment of cuttings.*—The percentages of established cuttings for all treatments are entered in the third section of Table I. The greatest effect was due

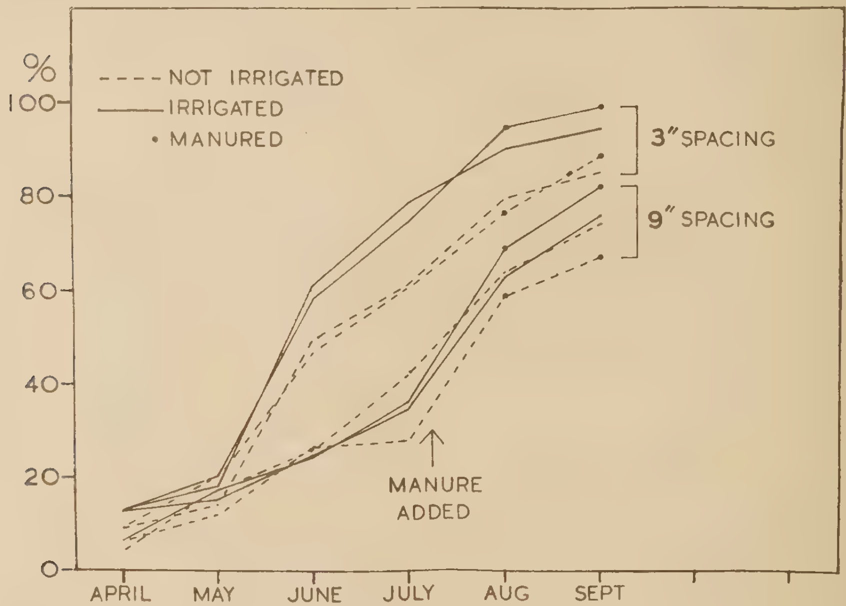

FIG. 4.

Irrigation and spacing experiment (loam soil), 1944-45. Graphical presentation of light intensity measurements, expressed as percentage of incident light absorbed by the foliage, throughout the growing season. All plots were recorded, the graphs being constructed from treatment means.

to spacing, with a significantly larger number of cuttings growing at the 3 in. spacing ($P < 0\cdot001$). Further, there was a beneficial effect of irrigation (irrigated 67·3 per cent., non-irrigated 58·3 per cent., $P < 0\cdot05$) ; but the manurial effect was not significant for the reason that by July 24th, when nitrogen was first applied, establishment had been completed.

(iv) *Growth records. Data of dry weight.*—Typical cuttings were photographed at harvest and the appearance of irrigated and manured plants is shown in Plate II. The great effect of spacing is apparent, wide spacing leading to very vigorous growth. The plants shown here should be compared with those in Plate I, when it will be seen that the irrigated cuttings in the present experiment, in spite of their vigour under wide spacing, approximate in form more to the typical plants on the loam than to

those on the lake soil, with branching which is much less free. The highly branched type of growth associated with the lake soil has thus not been induced in plants growing on loam either by irrigating or manuring.

The weight data are presented, and their statistical analyses summarized, in Table V, from which it will be seen that spacing has highly significant effects on total dry weight, shoot-root ratio, and ratio of secondary to primary shoots, but not on the ratio of side roots to basal roots. The widely spaced plants attained much greater size, had higher shoot:root ratios, and relatively more secondary shoot growth. The only other significant effect was that of irrigation on shoot:root ratio, this treatment increasing the proportion of shoot. Certain other tendencies suggested by the data should, however, be noted. With close spacing the irrigated and manured plants gave somewhat higher weight values, particularly for the shoot, in accord with the light intensity records (text Fig. 4). As regards root growth, spacing had no influence on the proportion of side roots, but the F value for irrigation was 7·29 and approached $P < 0{\cdot}05$. The interesting fact is, however, that the irrigated plants had the smaller proportion of side roots, the reverse of the natural effect between lake soil and loam (Table III). Finally, though the effect of irrigation on the degree of branching is insignificant, the results suggest that, with the close spacing particularly, the highly watered plants were, if anything, less branched, not more so, than those not watered ; this is again the reverse of the natural soil characteristics. It is quite evident, however, that the general form of the plants on the loam was unchanged by frequent irrigation.

DISCUSSION.

These experiments were undertaken primarily to study the effect of spacing on the establishment and growth of hardwood cuttings of the Myrobalan B plum rootstock. These two aspects of the problem are to a large extent independent, thougl. it is possible that the reactions of the cuttings occurring during the period of establishment may have a predetermining effect on later growth, and thus profoundly influence the final form of the plant. The evidence presented in this paper is derived from the characteristics of the plants at the end of one season's growth, no measurements having been made during the course of this growth.

The outstanding feature was the marked effect of soil type on the final form of the established plant, which concerned both root and shoot development. On the soil of the lake bed the root system was characterized by a more pronounced development of side roots. Comparing the photographs reproduced in Plate I this differential distribution of roots is very apparent ; for on the loam the roots are predominantly basal, whereas on the lake soil there is a copious production of side roots as well. The latter soil may thus be regarded as the better rooting medium ; for, except at the closest spacing, a greater weight of roots is formed as compared with the loam, and the roots are more uniformly distributed, the cutting ending the year's growth with roots at all levels in the soil, and not only at a depth.

Unfortunately the greater production of side roots on the lake soil is accompanied by much greater branching of the shoots (see also Vyvyan and Garner, 1946), a very undesirable character for subsequent operations by the propagator. The tendency of the shoots to branch is, however, markedly influenced by spacing conditions, and the experiments have demonstrated that one of the most important effects of close

spacing is the relative suppression of secondary shoot growth. This effect is clearly shown by the reduction in ratio of secondary to primary shoots, but the fact that there is a significant interaction of spacing with soil type indicates a far better control of branching by close spacing on the loam. On the lake soil the ratio of secondary shoots to primary shoots always exceeds two, and in the first experiment it averaged as much as four even for the close spacings (Table III, item 3).

From the experiments that have so far been performed it is not possible to make generalizations of wider application than those already stated. The results presented in text Fig. 2, for instance, indicate that considerable variations in the behaviour of cuttings on the two soil types may occur in different seasons. The results for 1944-45 are particularly striking in this respect. With close spacing the data are quite unequivocal, for the amount of shoot growth made is characteristic of the soil type, and independent of the season. In contrast, the response to variable spacing varies very greatly, and not in any way systematically. An analysis of this seasonal effect will not be undertaken here, but it is certainly a factor demanding further work. The important fact disclosed is that growth with narrow spacing is similar in the two seasons studied, and this gives some assurance that the beneficial effect of close spacing will be found in seasons other than those as yet observed.

It may be premature to speculate on the soil characters responsible for the different behaviour of the cuttings on the loam and lake soil. The one experiment so far performed was designed to test the effect of water supply. Judging from the results in Table II it would appear that the conditions under which the water demand on the soil is greatest leads to the maximum relative difference in shoot growth on the two soil types, the lake soil giving much the greater growth of shoots. It was natural to infer that this was a matter of water supply though the possibility of nutritive factors was not overlooked. It was to test this simple hypothesis that the third experiment reported was undertaken, in which the water supply in the loam was increased and maintained at a high level by irrigation throughout the growth period. The experiment showed, however, no significant effect of irrigation in spite of the usual highly significant spacing effect; there is thus no evidence that the differences in the two soils can be attributed merely to water supply. Further, and quite unexpectedly, the type of plant on the irrigated loam differed more from that on the lake soil than was the case for the non-irrigated treatments, branching (both of roots and aerial portions) being reduced by the higher water supply.

The complexity of the reactions is shown by the shoot:root ratios in relation to spacing on the two soil types. On the loam wider spacing encouraged shoot development in relation to roots, whereas on the lake soil the reverse was true. This interaction is significant, and so far as the loam is concerned the effect of wide spacing on shoot:root ratio was confirmed in the third experiment.

This result is one aspect of the factors determining form of the plant, which is dependent on the behaviour of the buds. The behaviour of the buds originally present on the cutting does not greatly differ, for in all cases two to three primary shoots are developed. The secondaries arising from buds on the primaries display, however, very great differences, both in relation to spacing and to soil type. By close spacing the greater tendency of secondary buds to develop on the lake soil plants cannot be eradicated. The difference between the buds on the original cutting and those on the primary shoots is in fact that in the former the terminal bud has been removed, while in the latter the primary shoot is complete. One may therefore

suspect, perhaps, that the factor of apical dominance plays a part, though the mechanism underlying this effect will demand further investigation. It should, however, be noted that the lake soil plants, as well as producing more highly branched shoots, are also characterized by a greater development of side roots ; and if apical dominance is in any way concerned, it raises the question of a possible effect on the root system acting in the opposite direction from that on the shoot. Teleologically this would be a very interesting correlation but, again, further work will be necessary to establish such a possibility. One point should, however, be noted, namely, that whereas both spacing and soil type markedly influence the degree of branching of the shoots, in the root system the relative amount of side root is strongly affected by the soil type, but not at all by spacing.

As already stated, the extent to which these soil effects are predetermined by the factors operating during establishment and early growth is quite unknown. The point of greatest interest is the specific effect of soil type on plant form, and these experiments have thrown little light on this question. It may well be that the physical characters of the soil are very important. It is known that the establishment of the cutting is affected by its anchorage in the soil, which is one of the advantages of using long cuttings sunk deeply ; thus the disturbance of the cutting mechanically by frost or wind will, in fact, have different effects on the loam and lake soils. It is planned to carry out experiments to investigate these effects, and particularly to separate the factors operating during establishment and subsequent growth by systematic transplantation from one soil to the other. The direct effect of the immediate environmental condition on the two soils during early establishment will also receive attention.

Finally, it can be said that the experiments described above have demonstrated quite conclusively the value of close spacing for the production of Myrobalan B plum rootstocks suitable for budding or grafting, from hardwood cuttings, the essential qualities being few primary shoots, a minimal degree of branching and absence of general coarseness of growth. The method of planting cuttings in short, closely spaced rows, arranged in beds, to allow hand cultivation from their ends, has been described elsewhere (Garner and Hatcher, 1946).

SUMMARY.

1. Experiments to investigate the effect of spacing on the growth and form of young plants derived from hardwood cuttings of the Myrobalan B plum, *Prunus cerasifera* Ehrh., rootstock are described.

2. On two soils of contrasting features, a normal loam, and a highly specialized lake-bed soil, close spacing resulted in plants of smaller general size, with reduced branching, without reduction in the percentage establishment of the cuttings.

3. Marked effects, due to soil and seasonal climatic conditions, were also observed, the plants on the lake soil being considerably more branched than those on the loam.

4. Irrigation of the loam throughout the growing season failed to produce the much branched form of plant typical of the lake soil.

5. The horticultural value of these effects of spacing is considered.

ACKNOWLEDGMENTS.

The authors express their great appreciation of the interest which Professor F. G. Gregory has taken in these experiments, and of his invaluable help in interpreting the data.

The authors also acknowledge advice and assistance from various members of the East Malling Research Station Staff, particularly those of the Statistical Section, for designing the trials and carrying out analyses; of Dr. W. S. Rogers for advising on the installation of the soil-moisture meters; and of Dr. E. E. Cheesman for suggestions as to presentation. To Professor G. E. Blackman of Oxford University, and Dr. A. J. Rutter of the Imperial College of Science, thanks are extended for the use of the light measuring apparatus.

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(*Received* 7/5/47.)

PLATE I.

Inter-row spacing experiment, 1942-43.
tographs of sample cuttings; loam plants in upper row, lake soil plants in lower row. Groups from left to right correspond to the spacings 4½ in., 9 in., 18 in. and 27 in.

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Irrigation spacing experiment on loam, 1944-45.
Photograph of sample cuttings from plots which received both irrigation and manurial treatments; 3 in. spacing plants on the left, 9 in. spacing plants on the right.

VARIATIONS IN THE REACTION OF WHITE BURLEY TOBACCO TO THE TOMATO AUCUBA MOSAIC VIRUS AND TO SOME OTHER STRAINS OF TOBACCO MOSAIC VIRUS

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Over a number of years, Tomato Aucuba Mosaic virus maintained in Kondine Red tomato plants at Rothamsted has consistently produced necrotic local lesions when transmitted to White Burley tobacco plants. Systemic infection has also been obtained occasionally, when the symptoms have consisted of an acute necrosis of the young leaves, usually leading to early death of the plants.

In 1945, however, a different result was obtained ; inoculated tobacco plants developed no necrotic local lesions and no necrosis but, instead, always exhibited symptoms of systemic infection in the form of a general bright yellow mosaic. This behaviour was first noticed with inoculum suspected to contain this virus obtained from a naturally infected tomato plant sent to Rothamsted for diagnosis by Dr. J. H. Western. This plant was almost entirely chlorotic, the yellow mosaic being more extensive and intensive than that caused in tomato by the Rothamsted stock culture of Tomato Aucuba Mosaic virus. By serological tests and transmission to *Nicotiana glutinosa* the virus was identified positively as a strain of Tobacco Mosaic virus, but its reactions in White Burley tobacco suggested that it was not the usual Tomato Aucuba strain. It gave no necrotic symptoms, but induced chlorotic local lesions and systemic symptoms consisting first of a yellow vein-clearing and, later, of a vivid yellow blotchy mosaic.

It was thought at first that this behaviour was a property peculiar to the new isolate ; but this supposition proved to be unfounded when comparative tests were made with it and the Rothamsted stock culture of Tomato Aucuba Mosaic virus, for both viruses readily became systemic and produced only mosaic symptoms. To make sure that this stock culture had not altered during repeated transmissions over a long period, inoculum was taken from infected tomato leaves dried 10 years previously. The virus from this source also gave mosaic symptoms only, though samples taken from it at intervals during the previous years had always given necrotic local lesions and occasionally systemic necrosis in White Burley tobacco plants.

The new isolate and inoculum from the stock of Tomato Aucuba Mosaic virus at Rothamsted were transferred to fresh White Burley seedlings on several occasions during 1945 without producing any necrotic symptoms. In the spring of 1946, however, when further inoculations were made, both virus strains gave necrotic local lesions. Inoculum taken at this time from systemically infected White Burley plants showing mosaic but no necrosis, produced only necrotic local lesions when inoculated into healthy White Burley seedlings.

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No definite explanation of this fluctuating behaviour can be offered. At the time it was thought that some change in soil conditions or other environmental factors might be affecting the response, but the results described below suggest that genetic differences between the tobacco seed used at the different times was the most probable cause.

At the Cheshunt Experimental Station experience over a number of years with Tomato Aucuba Mosaic virus (derived originally from the same source as that used at Rothamsted) has been different. In White Burley tobacco it has consistently given systemic symptoms of the mosaic type except in 1939, when some of the inoculated plants developed necrotic local lesions only, while others showed a mottle, although the inoculum was the same for all and the inoculated plants were raised from the same source. It was then noticed that there was a slight difference in the form of the leaves of the plants giving the two reactions. Leaves developing necrotic local lesions tended to be broader and to have fewer lateral veins per unit length of mid-rib than those of plants in which systemic infection developed. This suggested that genetical differences between the host plants might be responsible for their difference in reaction towards the virus. If so, then the original plant or plants of White Burley from which the seed was obtained must have been heterozygous, and the two kinds of reaction a result of segregation. Home-saved seed has been used at Cheshunt for many years and no new strain of White Burley tobacco has been grown there with which crossing might have occurred. Since 1941 all the White Burley plants raised there have failed to develop necrotic local lesions with any of the strains of the Tomato Aucuba Mosaic virus.

It is known that different varieties of tobacco react differently with certain strains of Tobacco Mosaic virus. For example, Kunkel (1932) found that one of them, viz. Tomato Aucuba Mosaic virus (obtained from England), produced necrotic local lesions in some varieties, including Burley and Little Oronoca, whereas in the varieties Connecticut Seed Leaf, Macrophylla and Purpurea chlorotic local lesions and a systemic mottle were produced by the same virus. It is generally believed, however, that the reaction of one variety of host to one virus strain is constant under the same conditions, and that such reactions are of diagnostic value. As White Burley tobacco is used extensively in virus work as a test plant, it was thought worth while to test its reaction to certain strains of Tobacco Mosaic virus, raising the plants from seed obtained from different sources. Such tests were made both at Cheshunt and at Rothamsted, for it was possible that differences in soil or other growing conditions might also influence the results.

Three lines of White Burley seed were used, namely two of Judy's Pride, from Wisconsin and Beltsville Md., U.S.A., respectively, and a third from a root-rot resistant White Burley obtained from the U.S.A. in 1943 and propagated since then at Rothamsted. The viruses used were: the Cheshunt and the Rothamsted stock cultures of Tomato Aucuba Mosaic virus, the isolate from the tomato plant supplied by Dr. J. H. Western, and the Rothamsted and Cheshunt stock cultures of Tobacco Mosaic virus. All three viruses from tomato caused necrotic symptoms in tobacco plants of the same two lines; they differed only in the intensity of the yellowing they caused in tomato and tobacco plants of the third line, in which they became systemic; no further distinction need be made between them here.

The results of the tests are summarized in the accompanying Table, which shows that all three lines of White Burley tobacco developed the same type of

symptoms when inoculated with the Rothamsted culture of Tobacco Mosaic virus, whereas they showed two distinct types of reaction with Tomato Aucuba Mosaic virus and the Cheshunt culture of Tobacco Mosaic virus. The Judy's Pride White Burley from Wisconsin developed no necrotic symptoms when inoculated with any of the virus strains used (Plate I, Fig. 1), whereas Judy's Pride from Beltsville and the root-rot resistant line reacted necrotically with Tomato Aucuba Mosaic virus and with the Cheshunt culture of Tobacco Mosaic virus. Some plants gave necrotic local lesions only, others developed in addition a severe systemic necrosis (Plate I, Fig. 2), which sometimes led to the death of the plants (Plate II, Fig. 3). The percentage of plants that became systemically infected varied from 10-60 in different tests. The plants became increasingly resistant to systemic infection as they grew older, but the onset of systemic infection may be determined also by factors other than age.

Whether or not a strain of Tobacco Mosaic virus produces necrotic local lesions on White Burley tobacco plants is usually considered a point of diagnostic value in identifying the particular strain of virus concerned. The results described above, however, show that within one named variety of tobacco there may be differences of genetic constitution that determine whether or not any particular virus strain will give necrotic local lesions ; and it is clear that this reaction may have little

TABLE.

Reaction of three lines of White Burley tobacco to Tomato Aucuba Mosaic virus and two other strains of Tobacco Mosaic virus.

Line of White Burley Tobacco.	Virus strains.		
	Tomato Aucuba Mosaic virus.	Cheshunt Tobacco Mosaic virus.	Rothamsted Tobacco. Mosaic virus.
Judy's Pride, from Wisconsin.	No necrotic symptoms ; occasionally chlorotic local lesions.	No necrotic local lesions.	No necrotic local lesions.
	Systemic infection starts as a pronounced yellow vein-clearing, later developing into a bright yellow mottle.	Systemic symptoms start with vein-clearing, followed later by a green mottle with slight malformation.	Systemic symptoms start as vein-clearing, followed by a green mottle with blistering and malformation.
Judy's Pride, from Beltsville, and a root-rot resistant White Burley from Rothamsted.	Necrotic local lesions; the only symptoms on many plants, especially if well developed when inoculated.	Necrotic local lesions, the only symptoms on older plants.	No necrotic local lesions.
	Systemic symptoms on young plants are severe necrosis of the veins, consequent death of the older leaves and crinkling of the younger ones. Plants which survive, produce new growth showing a bright yellow mosaic.	Systemic infection on young plants consisting of severe necrosis of the veins, death of the older leaves and crinkling of the younger ones. Plants which survive produce new growth showing a green mottle.	Systemic symptoms start as vein-clearing, followed by a green mottle with blistering and malformation.

diagnostic value, even when a named line of White Burley tobacco, such as Judy's Pride, is specified. It is important that this variability should be realized, for it has probably been responsible for some of the contradictory results obtained by different workers purporting to be using the same combination of viruses and hosts. Such genetical differences would also be expected to influence the strains occurring in stock cultures of Tobacco Mosaic virus. This virus seems to be continually producing new strains (Bawden, 1943) and a change in the genetic constitution of the host which affects its reaction to certain virus strains will exert a selective influence on them. In particular, strains that produce necrosis will tend to be eliminated owing to death of the host tissues and will ultimately be superseded by strains that cause mosaic symptoms and readily become systemic. Thus, the continuous culture of Tobacco Mosaic virus in the Wisconsin Judy's Pride line of White Burley might be expected to result in the selection of a strain giving a different result from that produced by a culture of this virus in the root-rot resistant White Burley; for some strains that give local lesions in the latter produce systemic infection of the former. Such unsuspected selective action on the part of the host probably explains the differences in the behaviour of the stock cultures of Tobacco Mosaic virus maintained at Rothamsted and Cheshunt. At both Stations this virus has been maintained in White Burley tobacco plants for many years, but at Rothamsted the plants used react necrotically to strains that give only a systemic mosaic in the plants used at Cheshunt. The strains that now predominate in the Cheshunt culture all produce necrosis in the tobacco plants used at Rothamsted and could not have maintained themselves in competition with the strains predominating in the Rothamsted culture, which produce mosaic in the plants.

SUMMARY.

As a result of tests made with Tomato Aucuba Mosaic virus and two other strains of Tobacco Mosaic virus it was found that the tobacco variety "White Burley" exists in two distinct lines, which show only slight morphological differences in the leaf laminae. Plants from one of these lines develop necrotic local lesions when inoculated with Tomato Aucuba Mosaic virus and, occasionally, severe systemic necrosis. Plants from the other line develop a yellow mottle with the same inoculum. In the lines of White Burley tobacco in which Tomato Aucuba Mosaic virus induces necrosis, some other strains of Tobacco Mosaic virus also react necrotically. The possible consequences of these results in virus work are discussed.

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(*Received* 13/5/47.)

PLATE I.

FIG. 1.

Judy's Pride tobacco plant, from Wisconsin, 5 weeks after inoculation with Tomato Aucuba Mosaic virus, showing a bright yellow mosaic ; no local lesions, no necrosis.

FIG. 2.

Judy's Pride tobacco plant, from Beltsville, 10 days after inoculation with Tomato Aucuba Mosaic virus, showing necrotic local lesions and the beginning of systemic necrosis.

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PLATE II.

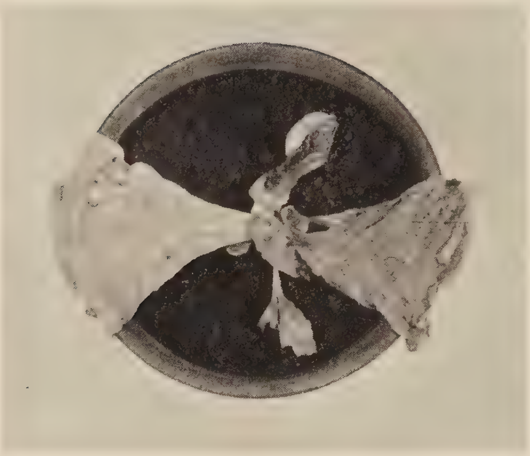

FIG. 3.

Root-rot resistant White Burley tobacco plant, 3 weeks after inoculation with the Cheshunt culture of Tobacco Mosaic virus, showing systemic necrosis.

THE EFFECTS OF TEMPERATURE AND GAS MIXTURES ON THE PRODUCTION OF VOLATILE SUBSTANCES BY APPLES DURING STORAGE

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SEVERAL methods have been used for the determination of the volatile substances produced by apples during storage. Gane (1935) and Kidd and West (1938) determined the total volatile substances by combustion in a furnace. Gerhardt and Ezell (1939) absorbed part of them in concentrated H_2SO_4. Nelson (1940) and Hansen and Christensen (1939) estimated only ethylene in the emanations from apples, and Southwick (1945) followed the procedure used by Gerhardt and Ezell.

Walls (1942) showed that the volatile substances produced by apples during storage could be separated into two portions, the odorous and the ethylene fractions, by absorption in concentrated H_2SO_4 and activated H_2SO_4* respectively; and this was confirmed by the present authors (1946).

The aim of the work now described (in which Wall's method was followed) was to find how the ratio of the two fractions was affected during storage by (1) temperature, and (2) gas mixtures.

Two temperatures, 15° and 5° C., were selected for the storage of apples in air, but one only, viz. 15° C., for storage in gas mixtures; these consisted of: (1) 5% O_2 + 95% N_2, and (2) 5% O_2 + 10% CO_2 + 85% N_2.

In addition to the estimation of volatile substances the respiration of the apples in air was determined at both temperatures.

EXPERIMENTAL.

A large sample of King Edward apples, carefully selected from a picking made on October 3rd, was divided into a number of sub-samples immediately on arrival at the laboratory. Each sample consisted of 11 hard and green apples and weighed approximately 1·5 kg. The samples were placed in large desiccators of 5-6 litres capacity through which air or gas flowed, at the rate of 3 litres an hour, into absorption apparatus as follows:

(1) For respiration measurements at 5° and 15° C., air from outside the building was drawn through a flowmeter, scrubbed free from CO_2 by passing over soda lime, and humidified by bubbling through 8 per cent. KOH solution. The stream of air then passed through the desiccator containing the apples and finally through Pettenkofer tubes, in which the CO_2 of respiration was absorbed by standard bartya solution. The tubes were changed at intervals according to the rate of CO_2 production, the baryta being titrated with standard HCl in the usual way. Analyses on three successive days of the air stream issuing from the desiccator, showed that the CO_2 content was 0·3 per cent. and that there was no accumulation of CO_2 in the desiccator.

* 2% w/v $Ag(SO_4)_2$, in concentrated H_2SO_4.

(2) For the estimation of the volatile substances emanating from apples stored in air at 5° and 15° C., air from outside the building was drawn through a flowmeter, over the apples in the desiccator and then through two Dreschel bottles with sintered spoons containing 50 cc. of (*a*) concentrated H_2SO_4 and (*b*) activated H_2SO_4. The acids were changed at intervals of 7-14 days and the carbon content of the absorbed volatile substances was determined by the wet combustion method of Raistrick (1931).

(3) When the apples were treated with gas mixtures (which were contained in high pressure cylinders), the gas was released through a needle valve and passed through the flowmeter. It was then humidified to 90 per cent. R.H. by bubbling through H_2SO_4 (1·124 sp.g., at 10° C.) and passed through the desiccators to the Dreschel bottles containing concentrated H_2SO_4 and activated H_2SO_4. The acids were changed at 7-14 day intervals and the carbon content determined.

RESULTS.

The results, expressed as ml. of CO_2 per 10 kg. per hour, are shown in Figs. 1 to 3. In addition, the ratios of the ethylene to the odorous fractions are given in Table I,

Table I.

Ratio of ethylene to odorous constituents (expressed as CO_2) in the volatile products from stored apples.

Stored at 5° C. in		Stored at 15° C. in					
Air.		Air.		5% O_2 + 95% N_2.		5% O_2 + 10% CO_2 + 85% N_2.	
Days.	Ratio.	Days.	Ratio.	Days.	Ratio.	Days.	Ratio.
3·5	1·4 : 1	3·5	1·5 : 1	—	—	—	—
10·5	2·6 : 1	10·5	1·5 : 1	—	—	10·5	0·6 : 1
17·5	1·8 : 1	17·5	0·9 : 1	17·5	2·1 : 1	17·5	1·3 : 1
24·5	1·5 : 1	24·5	0·8 : 1	24·5	3·1 : 1	25·5	1·4 : 1
31·5	1·4 : 1	31·5	0·7 : 1	31·5	2·0 : 1	35·5	1·7 : 1
41·5	1·2 : 1	42·0	0·6 : 1	40·5	2·2 : 1	46·5	1·0 : 1
55·5	1·3 : 1	56·0	0·6 : 1	55·0*	0·7 : 1	61·5	0·7 : 1
71·5	1·6 : 1	72·5	0·3 : 1	72·0	0·4 : 1	76·0	0·8 : 1
106·0	0·8 : 1	89·0	0·3 : 1	88·5	0·2 : 1	90·0	0·8 : 1
125·0	0·6 : 1	—	—	106·0	0·2 : 1	106·5	0·4 : 1
—	—	—	—	126·0	0·03 : 1	126·5	0·2 : 1
—	—	—	—	—	—	144·0	0·1 : 1
—	—	—	—	—	—	163·0	0·08 : 1

having been determined at the mean of each period for which determinations of carbon were made.

Scald appeared on most of the fruits, and in many cases there was partial rotting; but observations were continued for some time after this occurred, so that the effect of rotting on the production of volatile substances could also be followed. In one case only, viz. at 15° C. in air, was one rotted apple replaced by a sound one which had been stored under similar conditions.

DISCUSSION.

Figs. 1 and 2 show respiratory activity at 15° C. and at 5° C. in air together with the corresponding production of volatile substances at these temperatures.

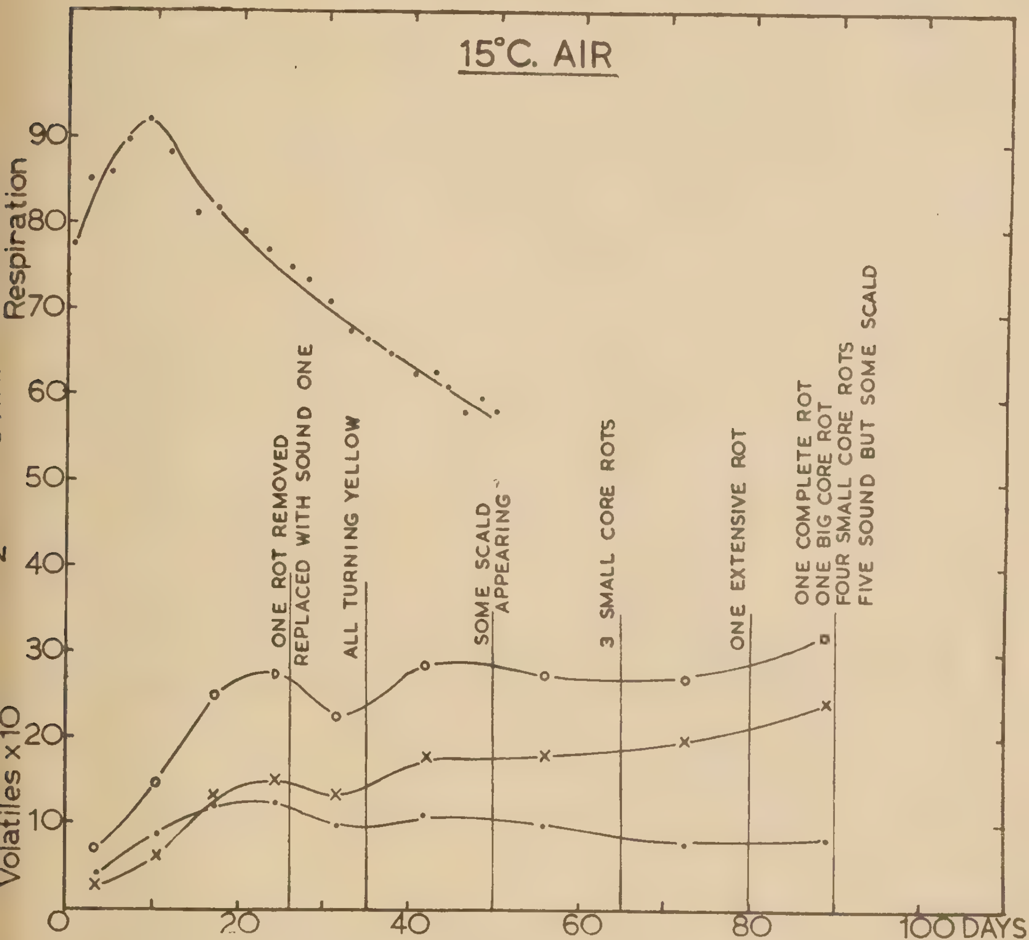

FIG. 1.

Production of CO_2 and volatile substances by King Edward Apples stored in air at 15° C.

●———● Ethylene fraction
×———× Odorous fraction
o———o Ethylene + odorous

Expressed as ml. CO_2 per 10 kg. per hour.

At 15° C. the climacteric rise begins at once and ends after 10 days storage, but the maximum production of ethylene occurs about 15 days later. The ethylene fraction is slightly greater than the odorous one at the beginning of storage, but after about three weeks begins to fall, whilst the odorous fraction continues to rise as the apples deteriorate, and is finally—after about 12 weeks—three times greater than

the ethylene one. The slight fall and subsequent rise in both fractions between the 25th and the 40th days may have been caused by the replacement of one rotted apple by a sound one ; but this is unlikely, since a similar fall and rise occurred when the apples were stored in the gas mixture (5% O_2 + 95% N_2) in which there was no replacement of any fruits.

At 5° C. there is a preliminary fall in the rate of respiration ; the climacteric rise begins 12 days after the beginning of storage and reaches a maximum approximately 55 days later. The ethylene fraction reaches its peak at about the same

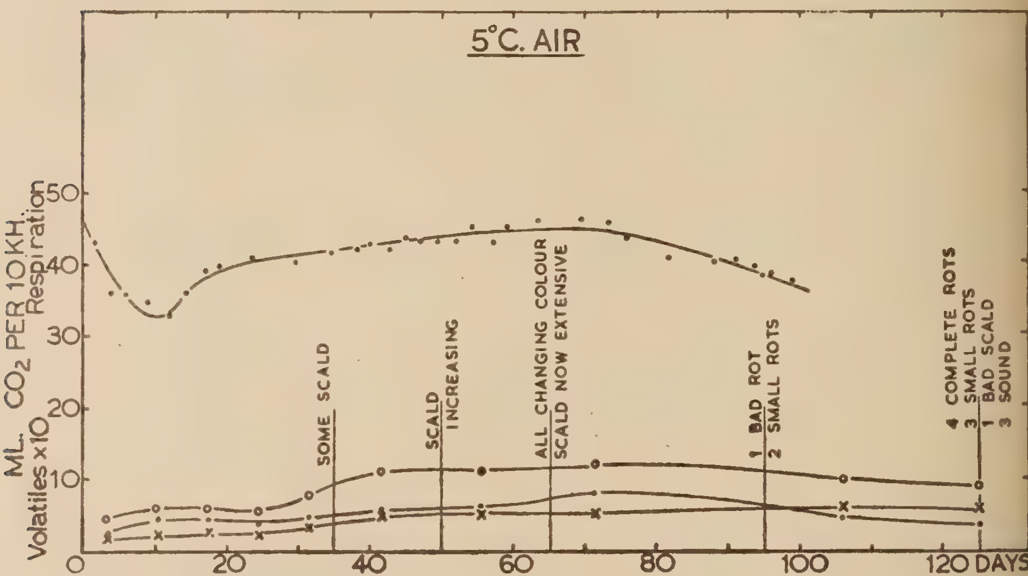

FIG. 2.

Production of CO_2 and volatile substances by King Edward Apples stored in air at 5° C.

●———● Ethylene fraction
×———× Odorous fraction
o———o Ethylene + odorous
Expressed as ml. CO_2 per 10 kg. per hour.

time and subsequently decreases, while the odorous fraction continues to rise gradually until the conclusion of the experiment on the 125th day.

During the period of the climacteric rise at 15° C. in air, the ratio of the ethylene to the odorous fractions is approximately 1·5 : 1 (see Table I). At 5° C., where the climacteric rise begins 10 days after the apples were put into store and continues until about the 70th day, the average ratio of the fractions is also 1·5 : 1. Although the total of volatile substances produced is greater at 15° C. than at 5° C., the ratio of the two fractions during the climacteric rise is therefore apparently not affected by temperature change within this range. When the post-climacteric phase ensues, the proportion of ethylene to odorous constituents becomes less than 1, and falls continuously as the fruit deteriorates.

Figs. 3*a* and 3*b* show the production of volatile substances during the storage of apples in the two gas mixtures. Fig. 3*a* illustrates the production of volatile

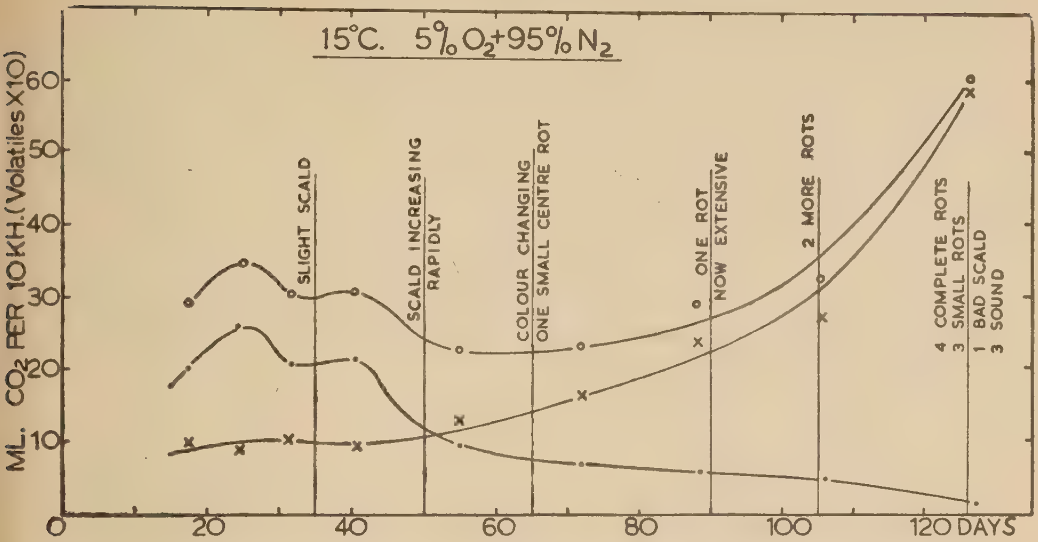

FIG. 3*a*.

Production of volatile substances by King Edward Apples stored in an atmosphere containing 5% O_2 + 95% N_2.

●———● Ethylene fraction
×———× Odorous fraction
o———o Ethylene + odorous

} Expressed as ml. CO_2 per 10 kg. per hour.

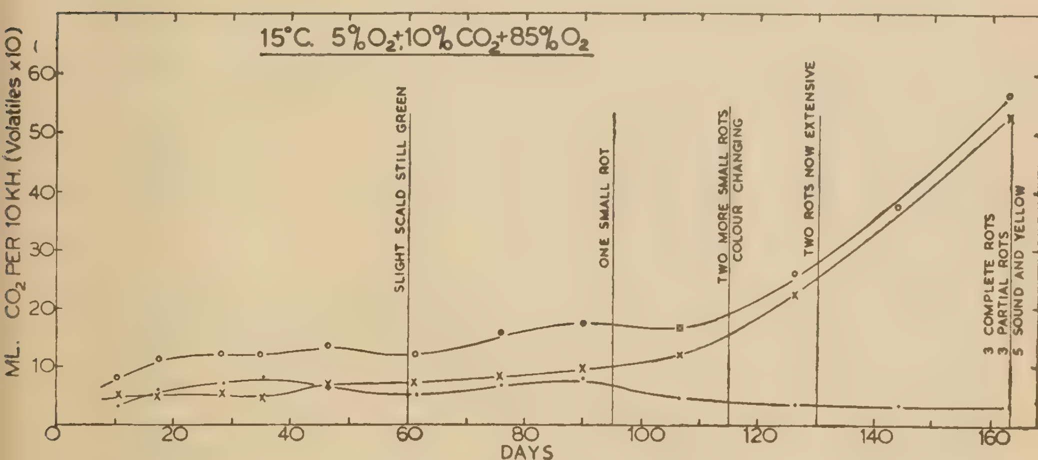

FIG. 3*b*.

Production of volatile substances by King Edward Apples stored in an atmosphere containing 5% O_2 + 10% CO_2 + 85% N_2.

●———● Ethylene fraction
×———× Odorous fraction
o———o Ethylene + odorous

} Expressed as ml. CO_2 per 10 kg. per hour.

substances produced in a gas mixture containing 5% O_2 + 95% N_2. The reduction of the O_2 content of the storage atmosphere to 5 per cent. causes a marked increase in the ethylene fraction, and a decrease in the odorous one, so that for the first 40 days of storage the ratio of ethylene to odorous constituents averages 2·5 : 1 (Table I). After 50 days in store, scald increases rapidly, rots appear and the colour of the fruit changes from green to yellow. There is then a rapid decline in ethylene production accompanied by a sharp rise in that of the odorous substances.

Fig. 3*b* shows that the effect of a gas mixture consisting of 5% O_2 + 10% CO_2 + 85% N_2 on the volatile substances is to lower the amounts produced, although the same falls and rises in the ethylene curve can be observed as are shown in Fig. 1 and Fig. 3*a*. The average ratio of the ethylene to the odorous constituents is above 1 for the first 46 days and then declines, slowly at first, and rapidly later when rots are present. As in the other gas mixture, the fruit changes colour when the ethylene fraction begins to fall.

SUMMARY.

(1) Of the volatile substances emanating from samples of King Edward apples stored in air at 15° C. and at 5° C., the ethylene fraction is produced, both during the climacteric rise and for some days after it, in greater proportion than the odorous fraction ; and the average ratio of the two fractions is similar for both temperatures until the climacteric peak is reached. For the remainder of the storage period, the rate of ethylene production falls, while that of the odorous fraction increases. This increase appears to be influenced by advancing rots.

(2) When the percentage of O_2 present in the storage atmosphere is reduced from 20 to 5 at 15° C., ethylene production is greatly increased for the first 40 days, and the subsequent decline is more rapid than in air at the same temperature. Corresponding with the greater production of ethylene, the production of the odorous constituents is reduced.

(3) If the CO_2 in the storage atmosphere is increased to 10 per cent. and the O_2 is reduced to 5 per cent., the rate of production of both fractions is considerably lowered.

(4) Changes in colour of the fruit from green to yellow take place during the decline in ethylene production in every case.

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VARIATION IN MAGNESIUM AND POTASSIUM CONTENT OF INDIVIDUAL LEAVES FROM MINERAL-DEFICIENT APPLE SHOOTS

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INTRODUCTION.

Work reported previously (1) has shown that apple leaves of the current season's growth vary in composition with their position on the shoot, and that variations which may occur in the leaves of the same leader are considerably affected by the relative availability of different minerals to the plant as well as by the age of the shoot.

Whereas previous analyses have been made only of composite samples, taken in sets of 3, 4 or 5 leaves from different parts of the leader, data are given in the present paper for individual leaves. These have been examined from a number of leaders, some of which came from trees showing mineral deficiencies and others from trees in orchards in which no visible symptoms of deficiency diseases have so far made their appearance.

EXPERIMENTAL.

A titan-yellow method (2), sensitive to 0·0005 mg. Mg, was used for magnesium, and a modification of the cobalti-nitrite method for potash.

SAMPLING.

Leaders to be compared were taken at approximately the same time of the year. Small top leaves under one inch in length were discarded. Petioles were removed before analysis.

RESULTS.

(a) *Magnesium.*

Magnesium figures are given in Table I for individual leaves on a number of leaders, collected in April, 1941, of the varieties Jonathan and Sturmer. All but one of these apple shoots were showing some defoliation due to magnesium deficiency, and the remaining leaves to the top of the shoot were analysed. The Nelson Sturmer was from an area rather low in potash but showing no scorch due to deficiency. Only leaves down to the 15th from near the top were analysed, those below and the small ones at the top being discarded.

Scorch due to magnesium deficiency was present on leaves ranging in magnesium content from 0·05 to 0·16 per cent. MgO (Table I). The leaves of all leaders showed a gradual rise in magnesium content as their position ascended towards the top of the shoot. The variation found from leaf to leaf on one and the same shoot emphasizes the importance of employing a consistent method of sampling for analytical purposes. A sample taken from the lower scorched leaves would give,

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TABLE I.

Magnesium Content of Individual Leaves of Apple Shoots. Results expressed as MgO per cent. on a dry matter basis.

Leaf position.	Jonathan (1) Braeburn. 10/4/41	Jonathan (2) Braeburn. 10/4/41	Sturmer (1) Braeburn. 10/4/41	Sturmer (2) Braeburn. 10/4/41	Sturmer Annesbrook 18/4/41	Sturmer Nelson. 22/4/41
Lowest leaf	0·09*	0·07*	0·05*	0·07*	0·12*	0·32
2nd ..	0·11*	0·09*	0·12	0·07*	0·10*	0·30
3rd ..	0·12*	0·13*	0·07*	0·11	0·09*	—
4th ..	0·12	0·16*	0·18	0·14	0·07*	0·25
5th ..	0·15	0·20	0·17	0·13	0·13	0·30
6th ..	0·14	0·18	0·22	0·18	0·12	0·32
7th ..	0·23	0·23	0·25	0·20	0·18	—
8th ..	0·22	0·22	0·25	0·25	0·20	0·35
9th ..	0·23	0·25	0·35	0·28	0·22	0·50
10th ..	0·22	0·28	0·38	0·27	0·25	0·56
11th ..	0·27	0·33	—	0·38	0·35	0·66
12th ..	0·27	0·96	0·53	0·46	0·38	0·99
13th ..	0·33	—	0·83	0·48	0·46	1·74
14th ..	0·28	—	0·90	0·83	0·61	2·77
15th ..	—	—	—	—	0·81	2·93

* Leaves showing blotching due to magnesium deficiency. In all leaders where leaf-scorch was showing, some defoliation had already taken place.

as typical of these magnesium-deficient leaders, a figure in the vicinity of 0·10 per cent. MgO, whereas if unscorched leaves were sampled—even if four or five of the upper leaves, having a relatively high magnesium content, were avoided—the magnesium content would be nearer 0·20 per cent. MgO. It has been shown (1) that magnesium deficiency can be detected early in the season, as it is indicated by the low magnesium content of the lower leader leaves ; and it is suggested that leaves from the lower half of the shoot taken late in December or early in January, before defoliation has started, may give the most useful indication of magnesium status under New Zealand conditions.

Results are given in Table II for individual leaves on a number of leaders of Cox's Orange and Sturmer varieties, some of which were normal and some showed typical symptoms of magnesium or potassium deficiency. The leaders were collected late in February or early in March, 1944, and still retained all their leaves. The results are expressed diagrammatically for the Cox's Orange samples in Fig. 1.

It will be seen from Table II that from the bottom to the top of the shoot the magnesium content of the leaves starts with a minimum in the lower leaves and thereafter rises gradually until highest values are reached (except in the Sturmer leader F) in the top leaves. Differences between the leaders tend to be less marked in the leaves near the growing point, even those of the magnesium-deficient leader C in that position approaching the normal ones in magnesium content. The leaves of the potash-deficient leaders D, E and F are probably abnormally high in magnesium, as a reciprocal effect due to their low potassium content. Potassium-deficiency symptoms were more severe in the leaves of leaders D and F than in those of E. In the one magnesium-deficient shoot examined (C), leaves showing visible

deficiency symptoms contained from 0·07 to 0·12 per cent. MgO. It is of interest to note that in the two apparently normal leaders A and B, the leaves over more than half their length had a content of MgO of less than 0·40 per cent., the figure given as the critical one by Wallace (3).

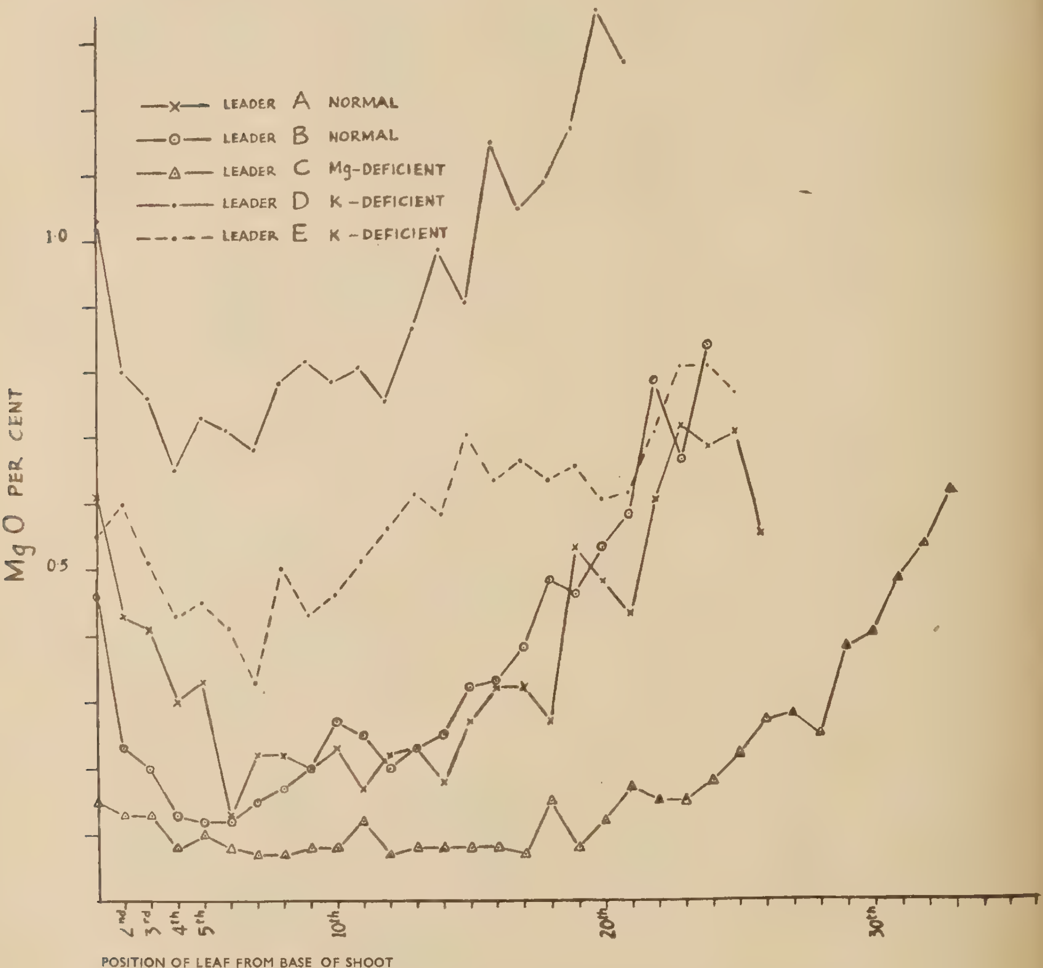

FIG. 1.

Magnesium content of individual leaves of apple leader shoots.

(*b*) *Potassium.*

Similar data for potassium are given in Table III and Fig. 2. The top two leaves of leader D were too small and too low in potassium to give a reliable result. Potassium figures are not available for leader E.

The leaves of the potassium-deficient leader D, compared with those of the three others of the same variety, were consistently low in potassium and showed little variation with position along the length of the shoot. In Sturmer F the

TABLE II.

Magnesium Content of Individual Leaves of Apple Shoots. Results expressed as MgO per cent. on a dry matter basis.

Leaf position.	Normal.		Magnesium -deficient.	Potassium-deficient.		
	Cox's Orange (A) Riwaka. 24/2/44	Cox's Orange (B) Waimea West. 7/3/44	Cox's Orange (C) Tasman. 7/3/44	Cox's Orange (D) Stoke. 7/3/44	Cox's Orange (E) Stoke. 7/3/44	Sturmer (F) Stoke. 7/3/44
Lowest leaf	0·61	0·46	0·15	1·03*	0·55*	1·14*
2nd ..	0·43	0·23	0·13	0·80*	0·60*	1·21*
3rd ..	0·41	0·20	0·13	0·76*	0·51*	0·85*
4th ..	0·30	0·13	0·08	0·65*	0·43*	0·63*
5th ..	0·33	0·12	0·10	0·73*	0·45*	0·56*
6th ..	0·13	0·12	0·08*	0·71*	0·41*	0·48*
7th ..	0·22	0·15	0·07*	0·68*	0·33*	0·66*
8th ..	0·22	0·17	0·07	0·78*	0·50*	0·81*
9th ..	0·20	0·20	0·08*	0·81*	0·43*	0·66*
10th ..	0·23	0·27	0·08*	0·78*	0·46*	0·71*
11th ..	0·17	0·25	0·12*	0·80*	0·51*	0·66*
12th ..	0·22	0·20	0·07	0·75*	0·56*	0·71*
13th ..	0·23	0·23	0·08	0·86*	0·61*	0·81*
14th ..	0·18	0·25	0·08	0·98	0·58*	0·73*
15th ..	0·27	0·32	0·08	0·90	0·70*	0·66
16th ..	0·32	0·33	0·08	1·14	0·63	0·86
17th ..	0·32	0·38	0·07*	1·04	0·66	0·83
18th ..	0·27	0·48	0·15	1·08	0·63	0·83
19th ..	0·53	0·46	0·08	1·16	0·65	0·83
20th ..	0·48	0·53	0·12	1·34	0·60	0·83
21st ..	0·43	0·58	0·17	1·26	0·61	0·99
22nd ..	0·60	0·78	0·15	—	0·70	1·03
23rd ..	0·71	0·66	0·15	—	0·80	1·19
24th ..	0·68	0·83	0·18	—	0·80	—
25th ..	0·70	—	0·22	—	0·76	—
26th ..	0·55	—	0·27	—	—	—
27th ..	—	—	0·28	—	—	—
28th ..	—	—	0·25	—	—	—
29th ..	—	—	0·38	—	—	—
30th ..	—	—	0·40	—	—	—
31st ..	—	—	0·48	—	—	—
32nd ..	—	—	0·53	—	—	—
33rd ..	—	—	0·61	—	—	—

* **Shows leaf scorch due to deficiency of Mg or K.**

distribution was similar, though the potassium status was a little higher. No leaf of these potassium-deficient leaders, D and F, contained more than the equivalent of 0·80 per cent. of K_2O. The potassium in the leaves of the two apparently normal

Cox's Orange leaders, A and B, was more than twice as much as that in those of leader D. There were indications of a slight decrease in the leaves towards the top of the shoot. In the leaves of the magnesium-deficient leader C, the potassium was less

TABLE III.

Potassium Content of Individual Leaves of Leader Shoots. Results expressed as K_2O per cent. on a dry matter basis.

	Normal.		Magnesium-deficient.	Potassium-deficient.	
Leaf position.	Cox's Orange (A). Riwaka. 24/2/44	Cox's Orange (B). Waimea West. 7/3/44	Cox's Orange (C). Tasman. 7/3/44	Cox's Orange (D). Stoke. 7/3/44	Sturmer (F). Stoke. 7/3/44
Lowest leaf	1·6	1·2	2·5	0·45*	0·50*
2nd ..	1·7	1·6	2·3	0·50*	0·55*
3rd ..	1·6	1·6	2·1	0·35*	0·55*
4th ..	1·4	1·6	2·5	0·25*	0·75*
5th ..	1·5	1·8	1·7	0·40*	0·55*
6th ..	1·4	1·7	2·0*	0·35*	0·55*
7th ..	1·5	1·7	2·1*	0·60*	0·60*
8th ..	1·6	1·7	2·4	0·40*	0·65*
9th ..	1·4	1·7	1·8*	0·55*	0·75*
10th ..	1·5	1·5	2·0*	0·50*	0·65*
11th ..	1·2	1·4	1·6*	0·45*	0·70*
12th ..	1·3	1·5	1·0	0·50*	0·80*
13th ..	1·1	1·5	1·4	0·45*	0·60*
14th ..	1·2	1·4	1·6	0·50	0·60*
15th ..	1·1	1·3	1·2	0·50	0·65
16th ..	1·0	1·3	1·5	0·45	0·65
17th ..	1·1	1·3	2·1*	0·45	0·60
18th ..	1·1	1·1	1·8	0·40	0·65
19th ..	1·1	1·0	1·9	0·40	0·65
20th ..	1·3	1·4	1·9	—	0·70
21st ..	1·2	1·0	1·8	—	0·60
22nd ..	1·2	1·3	1·9	—	0·50
23rd ..	1·4	1·2	1·7	—	0·35
24th ..	1·1	1·1	2·0	—	—
25th ..	1·2	—	1·8	—	—
26th ..	1·8	—	1·7	—	—
27th ..	—	—	1·8	—	—
28th ..	—	—	1·7	—	—
29th ..	—	—	1·5	—	—
30th ..	—	—	1·4	—	—
31st ..	—	—	1·2	—	—
32nd ..	—	—	0·4	—	—
33rd ..	—	—	0·7	—	—

* Shows leaf scorch due to deficiency of Mg or K.

evenly distributed. The figures showed a decrease followed by a rise and then a sharp fall towards the top; but on the average they were considerably higher than in the leaves of normal shoots.

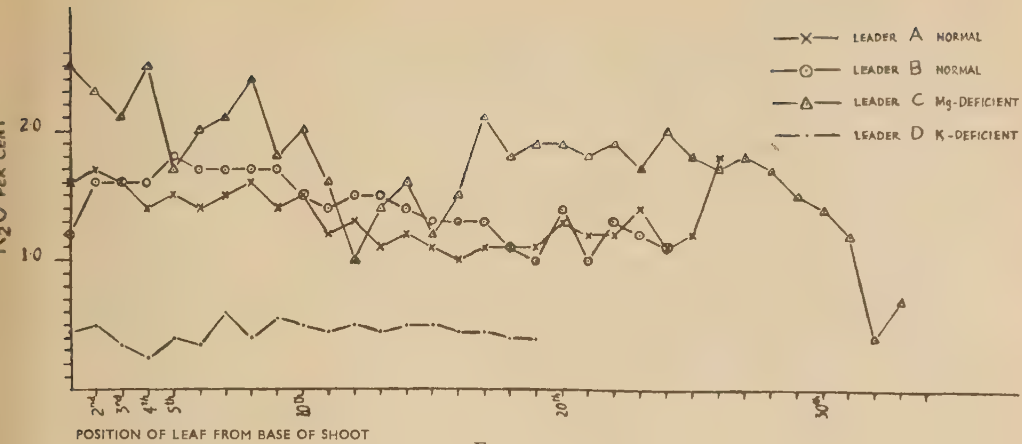

FIG. 2.

Potassium content of individual leaves of apple leader shoots.

DISCUSSION.

It must be emphasized that the distribution of magnesium and potassium in the leader leaves at any particular part of the season may not necessarily be typical of that at any other stage of growth. For instance, in the potassium-deficient leader D (Table III) the potassium varies only slightly with position of the leaf, but previous analyses have shown a very definite increase in potassium in the leaves towards the top of the shoot in a number of composite samples from the same locality taken earlier in the season (1). Similarly, the relatively high magnesium content of the top leaves in the same leader (Table II) appears to be an effect occurring late in the season (1). On the other hand, in the magnesium-deficient leader C, the slight decrease in magnesium in the leaves near the bottom of the shoot, and the rise to almost normal in those at the growing point (Table II), appear to be typical of leaves of magnesium-deficient leaders of this variety over the greater part of the season, though the amount of magnesium per leaf may change, particularly in leaves on the lower part of the leader. It is hoped to check results by further analyses of individual leaves and to obtain similar information for sets of leaders sampled at other times in the season.

The data again show that in the study of magnesium and potassium deficiencies, it is desirable always to secure samples of leaves from a definite part of the leader shoot, avoiding the top leaves.

Though there appear to be no figures available for all the individual leaves of the leader shoot, except those reported in this paper, Goodall has analysed by spectrographic methods a large number of leaves from different parts of the leader growth and spurs of apple trees grown at East Malling in England. His first published results (4) show no resemblance to the figures given in this paper and in a previous one from the Cawthron Institute (1). The figures for lime are much lower, and those for magnesium are such as might be obtained from samples with magnesium-deficiency, though the presence of scorch is not mentioned. There is

no increase of magnesium in the top leaves, which appears to be a characteristic of magnesium-deficient shoots in the Nelson district. With low magnesium there is no correspondingly high potassium, such as would be expected. The fact that the mid-rib was excluded from Goodall's samples would not appear to explain these large differences. Goodall comments that his methods of analysis improved before his 1943 analyses were carried out (5), and the results of these show a closer relationship to those presented in this paper, although the apple varieties were for the most part different. Thus, low magnesium was accompanied by high potassium, and vice versa. There are, however, in the 1942-43 results no data on the variation with position on the leader shoot. Goodall's results in a third paper (6) show the same tendency as that found in the Nelson district of New Zealand, for differences in mineral intake by the plant to be less detectable in young immature growth than in more mature leaves.

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FRUIT SPRAYING TRIALS WITH CERTAIN RECENTLY-INTRODUCED FUNGICIDES

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In the category of the more conventional protectant fungicides the chief developments of the last decade have been in the utilization of a wider range of copper compounds and in improvements in the physical qualities of the sulphur suspensions (the so-called "colloidal" or wettable sulphurs). A newer series of sulphur fungicides has been provided by the derivatives of thiocarbamic acid. Further, the long-standing use of mercury for the fungicidal treatment of seeds and soil has recently been extended to foliage sprays by the development of new organic mercury derivatives (Roberts, 1946).

In contrast to these modifications of the established specifics—copper, sulphur and mercury—there has been introduced a number of fungicides containing neither metallic radicles nor sulphur. Included in this class are the substituted quinones and quinolines and the glyoxalidines.

In all the above-mentioned groups the application of the new fungicides has been directed to various ends, including mildew-proofing of textiles, fumigation of seedlings and plant injections. In this paper, only one category of uses will be considered—protectant sprays on deciduous fruit plants. Further, discussion will be confined to those materials that have reached the stage of assessment by large-scale field trials.

REVIEW OF PUBLISHED WORK.

Dithiocarbamates.

Included in this group are the dimethyldithiocarbamates of iron and zinc, tetramethylthiuram disulphide and di-sodium ethylene bisdithiocarbamate. General accounts of the use of dithiocarbamates as fungicides are given by McCallan (1946) and Barratt and Horsfall (1947).

Ferric dimethyldithiocarbamate (Fermate).

The majority of reports of field trials with Fermate for the control of fruit diseases are from U.S.A. On apples, Palmiter and Hamilton (1942) state that Fermate at 2 lb. per 100 gallons equals wettable sulphur for Scab control: the same authors (1945) record that it is also effective against Sooty Blotch. Stoddard and Heuberger (1943) claim that it has an eradicant action on Scab pustules. Chamberlain (1945), while placing lime sulphur (1-40 or 1-60) as generally the most effective Scab spray in Ontario, records that Fermate is very promising, particularly when used with lime and Orthex spreader. Daines and Hopperstead (1946) consider Fermate at 1½-2 lb. per 100 gallons comparable to wettable sulphur, but note an instance of russeting following a Fermate-lime application.

On pears, in Oregon, Kienholz and Childs (1945) record that Fermate gives consistently good control without injury and is compatible with oils.

The use of Fermate at ½-1 lb. per 100 gallons on cherries for the control of Leaf Spot and Brown Rot is advocated by Tisdale and Flenner (1942), and by Palmiter and Hamilton (1942, 1943, 1945). The use of ½ pt. cottonseed oil per 100 gallons is recommended to obviate visible residue.

Goldsworthy *et al.* (1943) record that Fermate controlled Scab and Brown Rot in peaches, but caused leaf spotting. Wilson and Scott (1943) state that Fermate at 1 lb. per 100 gallons controlled peach Brown Rot, Rust and Shot-hole.

Wilcox (1944) notes a better control by Fermate, 3 lb. per 100 gallons, than by Bordeaux mixture on cranberry rot caused by *Guignardia vaccinii.* Bailey and Sproston (1946) find Fermate promising for the control of a Sclerotinia disease of the fruit of blueberry.

Zinc dimethyldithiocarbamate (Zerlate, Zincate, Fuklasin).

Zerlate is recorded by Howard (1946) and Palmiter (1947) as causing leaf and fruit injury on apples. Fuklasin (containing 20 per cent. zinc dimethyldithiocarbamate) has been approved in Germany for use against apple Scab (Anon., 1944). Lewis and Thurston (1946) state that it is effective against Alternaria Rot of cherries.

Tetramethylthiuram disulphide (O.B.72, Pomarsol, Pomasol, Arasan, Tulisan, Thiosan, Tersan, Jap beetle spray).

Tetramethylthiuram disulphide has been widely tested both in Europe and in North America. On apples it was recorded by Loewel (1937) in Germany as equal to lime sulphur-lead arsenate or copper sprays in the control of Scab. In England, tests by Moore *et al.* (1939) and by Montgomery *et al.* (1942) have given inconsistent results, the material having been promising for Scab control in some years, but unsatisfactory in others. Pomasol was officially approved for use against Scab in Germany in 1944 (Anon., 1944).

Hadorn (1943) states that Pomarsol in Switzerland has proved outstanding for Scab control: it is less adhesive than lime-sulphur, but the spray residue protects untreated foliage by re-dispersal. Mulder (1946) records from Holland that Pomarsol, as a summer spray, gives better control than the sulphur suspensions, but has no effect on red spider.

In U.S.A. good results against apple Scab were noted by Tisdale and Flenner (1942), but Roberts (1942) noted that tetramethylthiuramdisulphide mixed with lime or lead arsenate was injurious to apple and cherry foliage. Stoddard and Heuberger (1943) claimed that Jap beetle spray had a marked eradicant action on leaf Scab infections. Daines and Hopperstead (1946) recommended Thiosan at ¾ lb. per 100 gallons against *Phoma pomi.*

On cherries, Palmiter and Hamilton (1943) showed that Jap beetle spray at 1 lb. per 100 gallons was effective against Brown Rot and Leaf Spot.

Disodium ethylene bisdithiocarbamate (Dithane, He175).

Dithane, first recommended by Dimond *et al.* (1943), has been used principally as a potato spray, but some records of tests on fruit in U.S.A. have been published. Daines and Hopperstead (1946) state that He175 at 0·5 to 1·8 lb. per 100 gallons, either alone or with zinc sulphate-lime, failed to control apple Scab. Palmiter and Hamilton (1945) record that Dithane was disappointing against Scab, but promising against cherry Leaf Spot and Brown Rot.

Organic mercurials.

Phenyl mercury triethanol ammonium lactate (Puratized N5).

Howard *et al.* (1944) record that 20 gm. Puratized N5 per 100 gallons gives equal or better control of apple Scab than 10 lb. wettable sulphur or 1½ lb. Fermate, and suppresses sporulation in Scab lesions. Daines and Hopperstead (1946) state that Puratized N5X, at 1 in 1,000 and 1 in 2,000, is as effective as 1½ per cent. lime sulphur in controlling Scab, and that the addition of oil did not increase damage. Hamilton and Mack (1947) described Puratized agricultural spray as a good but expensive Scab eradicant, which is injurious in some areas and not compatible with sulphur, lime, bentonite, summer oils, hard or dirty water. Lewis and Thurston (1946) say that it cannot be used after petal fall. Folsom (1946) states that Puratized N5-E, at 1/20 gallon to 100 gallons, was of little use against apple Scab in Maine.

(There is a general reluctance to employ mercury sprays on apples in the U.S.A. because no tolerance for mercury on fruit has been announced.)

Phenyl mercury chloride (the fungicidal ingredient of Mercurated arsenate: Venturicide).

Reports on this material have been published only in Britain. Montgomery *et al.* (1942) conclude that phenyl mercury chloride is highly fungicidal, but not entirely safe on apples. Shaw and Moore (1945) consider that phenyl mercury chloride at 0·005 per cent. on lead arsenate (Mercurated arsenate) gives roughly the same degree of apple Scab control as 1 per cent. lime sulphur.

Substituted quinones and quinolines.

Tetrachlorobenzoquinone (Spergon, Chloranil).

Few reports have been made of spray trials with Spergon on fruit. However, Palmiter and Hamilton (1943) record that it was inferior to Fermate or Tersan in controlling Brown Rot of cherries.

2:3-Dichloro-1:4-naphthoquinone (Phygon: U.S.R. No. 604).

Daines and Hopperstead (1946) state that U.S.R. 604 at 1 lb. per 100 gallons equalled 1½ per cent. lime sulphur in apple Scab control, but caused excessive injury when mixed with oil. Howard (1946) reports that Phygon at ½-1 lb. per 100 gallons controlled Scab satisfactorily, but slightly reduced photosynthesis. Lewis and Thurston (1946) record that it shows much promise, but is liable to injure apples and cherries. This opinion is confirmed by Mills (1947).

8-Hydroxyquinoline sulphate (Copper 8-quinolate; Carpinol, Cryptonol).

Deloustal (1943) noted that a paste containing hydroxyquinoline sulphate promoted callusing of apple wood after the excision of Canker lesions. Preparations of this material have been commercially recommended in France for apple Scab control, and it has been claimed that the fungicide is absorbed by the tree, on which it confers systemic resistance.

Powell (1946) rated copper 8-quinolate as a promising fungicide in tests against apple Scab in U.S.A., and Hamilton and Mack (1947) state that this material at 1 lb. per 100 gallons plus 1 pint of Orthex spreader gave excellent Scab control.

Lauryl isoquinolinium bromide (Isothan Q15).

Howard *et al.* (1944) report that Isothan Q15, at 1½ oz. per 100 gallons, gives an equal or better control of apple Scab than 10 lb. wettable sulphur or 3 lb. Fermate.

In the 1945 report of the Rhode Island Experimental Station ([Howard], 1945) Isothan Q15, at 1/5,000, is said to give an excellent control of Scab, to inactivate Scab lesions, to be compatible with lead arsenate and nicotine sulphate and not objectionable to the operator. At 1/2,000 it causes some spray injury. Daines and Hopperstead (1946) claim that it is comparable to the standard wettable sulphurs in Scab control and compatible with oil. Lewis and Thurston (1946) state, however, that Isothan Q15 was unsuccessful under Pennsylvanian conditions.

Glyoxalidine derivatives.

2-Heptadecylglyoxalidine (Heptadecyl imidazoline, Fungicide 341).

Thurston *et al.* (1946), as a result of a 5-year field test, showed that " 341 ", at 1 lb. per 100 gallons, was equal to standard lime sulphur and better than Fermate, 3 lb. per 100 gallons, in apple Scab control. It was less damaging than lime sulphur. Lewis and Thurston (1946) note that " 341 " was promising against apple Scab and cherry Leaf Spot and showed a prolonged residual effect. Harry *et al.* (1947) pointed out that if heptadecylglyoxalidine was melted and slurried in hot water to give a dispersible paste, the compound was hydrolysed and lost fungicidal power. Certain commercial samples which had been prepared by this method were tested in 1946 and gave poor results (Hamilton and Mack, 1947). Heptadecylglyoxalidine is compatible with acid lead arsenate, nicotine sulphate, hydrated lime and summer oil.

1-Hydroxyethyl-2-heptadecylglyoxalidine (Hydroxyethylheptadecyl imidazoline, Fungicide 337).

Thurston *et al.* (1946) showed that this compound was equal to " 341 " in apple Scab control, but caused some foliage injury at 3 lb. per 100 gallons.

EXPERIMENTAL.

The present paper summarizes the results of field trials carried out in 1944-47, mainly at Long Ashton, with certain of the organic fungicides referred to above and, in addition, with tetrachlornitrobenzene (Folosan DB 905) and salicylanilide (Shirlan). The trials were made principally on apples and black currants against Scab (caused by *Venturia inaequalis*) and Leaf Spot (caused by *Pseudopeziza ribis*) respectively. Other trials were made against Brown Rot of apples and plums, and one spray damage test was carried out on gooseberries.

Plots.

At Long Ashton.

Plot 23 (Apples).—This 8-acre plot was set out in 1935 on a 15-ft. square plant. The western half comprises 23 rows of 30 trees per row. The whole forms a solid block of the variety Worcester Pearmain with the exception of Laxton's Superb pollinators which constitute rows 1, 12 and 23, and trees 1, 8, 15, 16, 23 and 30 in each of the other rows. At the NW. corner of plot 23 is another block of Worcesters (sub-plot N) in 6 rows of 10 trees per row. The plot also includes 20-tree blocks of Stirling Castle and of Lane's Prince Albert, which were used in the present investigation for spray damage trials.

Plot 17 (Apples).—This 3-acre plot, consisting mainly of the varieties Lane's Prince Albert, Laxton's Superb and Worcester Pearmain, was described and figured by Kearns *et al.* (1945).

Plot 3 (Blackcurrants).—The bushes used comprised 32 rows of the variety Baldwin, 7 bushes per row. Fuller details are given by Marsh and Dickinson (1944).

The trials on plums were made on a few trees of the new variety Avon Cross which is very susceptible to Brown Rot (Spinks, 1946), and the gooseberries sprayed were of the variety Leveller.

IN WORCESTERSHIRE.

The trial carried out in Worcestershire was made on 30-year-old cordons of the apple Cox's Orange Pippin. A description is given by Bennett *et al.* (1944).

MATERIALS.

Ferric dimethyldithiocarbamate.—This was tested in three forms:

(*a*) Commercial Fermate, obtained from U.S.A. by courtesy of Messrs. Plant Protection, Ltd. This was said to contain 60 per cent. ferric dimethyldithiocarbamate and included a spreader. At 2 lb. per 100 gallons it readily formed a stable suspension in water.

(*b*) Commercially pure ferric dimethyldithiocarbamate, kindly supplied by Messrs. Robinson Bros., of Birmingham. For use as a spray, this material was compounded at Long Ashton with sulphite lye in the proportion 2 lb. dithiocarbamate, 0·2 gal. sulphite lye syrup, 1·8 gal. water. The lye was dissolved in the water, the fungicide stirred in and the whole ground for 1 hour in a ball mill.

(*c*) The same material from Messrs. Robinson Bros. was milled with ester salts, instead of sulphite lye, using 1 pint ester salts (an 11 per cent. solution of secondary alkyl sulphates) to 2 lb. of the dithiocarbamate.

Zinc dimethyldithiocarbamate.—This was supplied by Imperial Chemical Industries Ltd. as a dispersible powder containing 80 per cent. of the pure compound. The powder was used at 1¼ lb. per 100 gallons.

Tetramethylthiuram disulphide.—Tetramethylthiuram disulphide sprays were prepared from the commercially pure chemical by methods (*b*) and (*c*) described above. It was also supplied by I.C.I. Ltd. as a dispersible powder, containing 46 per cent. T.M.T.D.S., which was used at 2 lb. per 100 gallons.

Disodium ethylene bisdithiocarbamate.—This was obtained from U.S.A. by Chas. Lennig & Co. as a liquid product, Dithane D14, containing 25 per cent. of the thiocarbamate. It was used mixed with zinc sulphate and lime, as recommended by Barratt and Horsfall (1947) in the proportions: ½ gal. D14, 1 lb. zinc sulphate, ½ lb. hydrated lime, 100 gal. water.

Phenyl mercury chloride.—Phenyl mercury chloride, supplied by Messrs. Lunevale Products, was employed in four forms:

(*a*) Venturicide; a specially compounded batch containing 2·5 per cent. phenyl mercury chloride in a methyl cellulose wetter.

(*b*) Mercurated arsenate, containing 2·5 per cent. phenyl mercury chloride precipitated on lead arsenate, with a sulphite lye wetter.

(*c*) An experimental product with 2·5 per cent. phenyl mercury chloride precipitated on zinc oxide and having Estol H as wetter.

(*d*) Phenyl mercury chloride in Grade E oil emulsion, used only in winter treatment against Brown Rot of plums (see under).

Tetrachlorbenzoquinone.—The material used was a commercial sample of Spergon which was compounded with Estol H to prepare a spray containing 2 lb. Spergon and 3 pints Estol H per 100 gallons water.

Dichloronaphthoquinone.—A commercial sample of Phygon from the U.S. Rubber Co., stated to contain 98 per cent. 2:3-dichloro-1:4-naphthoquinone, was ground with sulphite lye in the proportion of 1 lb. Phygon to 3½ pints sulphite lye for 100 gallons of spray.

Tetrachlornitrobenzene.—This was used as Folosan DB 905, stated to contain 5 per cent. tetrachlornitrobenzene, at the rate of 2 lb. per 100 gallons.

8-Hydroxyquinoline sulphate.—Material of 8-hydroxyquinoline sulphate monohydrate (neutral) was provided by British Quinoline Ltd. 1 lb. of this was mixed with 4 oz. hydrated lime to make 100 gallons of spray.

Salicylanilide.—This was used as Shirlan A.G. containing Agral as a wetter, made up at a strength of 2 lb. per 100 gallons.

Heptadecylglyoxalidine.—This material was received from the Carbide and Carbon Chemical Corporation (N.Y.) in four forms:

(*a*) Fungicide 341: undiluted 2-heptadecylglyoxalidine.

(*b*) 341 " A ": containing 40 per cent. of the glyoxalidine with a small quantity of wetting agent dispersed on kaolin.

(*c*) 341 " B ": containing 33 per cent. of the glyoxalidine dispersed on kaolin.

(*d*) 341 " C ": a liquid preparation with 54 per cent. of the glyoxalidine as an acetate salt dissolved in isoproponal.

For spraying, all these materials were made up at the equivalent of 1 lb. of the glyoxalidine per 100 gallons. The liquid preparation 341 " C " was used at 1 quart per 100 gallons plus 2½ oz. hydrated lime to liberate the free base.

Hydroxyethyl-heptadecylglyoxalidine.—This was provided by the Carbide and Carbon Chemicals Corporation as undiluted 1-hydroxyethyl-2-heptadecylglyoxalidine (Fungicide 337). For use it was made into a paste with water and diluted to 1 lb. per 100 gallons.

Methods.

The majority of the spray treatments were applied using a power sprayer and 3-nozzle brooms, with 4/64 in. discs, at a pressure of 400 lb. per sq. in. In some instances, when only small quantities of the fungicides were available, barrow pumps or bucket pumps were used, but special care was taken throughout to ensure thorough application. For apple trees, 2½-3 gallons were applied per tree, and for black currants ¾ to 1 gallon per bush. Unless otherwise stated, all spraying was carried out under favourable conditions.

The field performances of the fungicides against *Pseudopeziza ribis* on black currants were evaluated by eye estimations of the degree of foliage retention per bush in the late summer, marked on an arbitrary scale of 0-10. Treatments were randomized in blocks, and the mean value for each treatment in each block was expressed as a percentage foliage retention.

In evaluating fungicides used against apple Scab, estimations of both leaf and fruit infection were made. Scab infection on leaves was treated by the method of matching leaves against diagrams showing 0, 5, 10, 15, etc., percentage scabbed

area. This method, more fully described by Kearns *et al.* (1945), made it possible to compute the mean percentage scabbed area per leaf for the sample taken. The method was extended in the 1947 counts for use on fruit, and diagrams were prepared

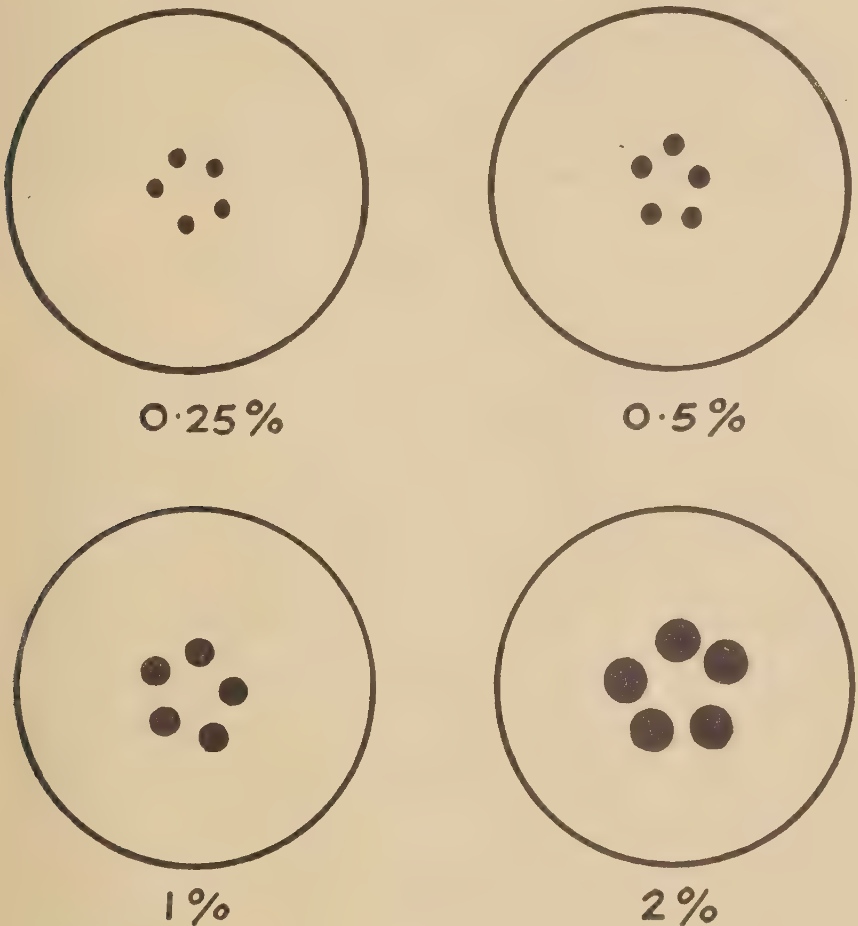

FIG. 1.

Scale for estimating percentage scabbed area on fruits.
For each diagram, the aggregate area of the five spots is stated as a percentage of the total surface area *of a sphere* of circumference shown.

(Fig. 1) showing aggregate scabbed areas as a percentage of the surface of an (assumed) spherical apple of the circumference shown. In earlier counts the apples were simply divided into two groups—clean and scabbed.

RESULTS.

Trials against Apple Scab.

Trials in 1945 using tetramethylthiuram disulphide and ferric dimethyldithiocarbamate.

In this trial, carried out on Plot 17 at Long Ashton, the two experimental sprays, both at 2 lb. per 100 gallons, were applied at the early pink, fruitlet and mid-summer stages, in comparison with lime sulphur used at the same times at 3 per cent. pre-blossom and 1 per cent. (twice) post-blossom. A full description of the trial is given by Kearns *et al.* (1945). Because of the destruction of the crop by frost, Scab was estimated on the leaves in August. Table I shows the mean percentage scabbed areas per leaf on the Worcesters and Superbs combined.

Table I.

Apple Scab control trial, 1945. Mean percentage scabbed areas per leaf.

Lime sulphur	$0 \cdot 28 \pm 0 \cdot 06$
Tetramethylthiuram disulphide ..	$0 \cdot 75 \pm 0 \cdot 13$
Ferric dimethyldithiocarbamate ..	$1 \cdot 23 \pm 0 \cdot 23$

Statistical analysis showed that both lime sulphur and the disulphide, on the figures given, were better than the dithiocarbamate, but the difference between lime sulphur and tetramethylthiuram disulphide (0·47) just failed to reach the level required (0·48) for significance at $P = 0 \cdot 05$.

The outstanding disadvantage shown by ferric dimethyldithiocarbamate was the disfiguring, black deposit left on the fruit. This residue was highly tenacious and remained apparent on the stored apples after picking.

Neither of the organic sulphur sprays, used alone, caused damage to Lane's Prince Albert and, in certain blocks where the fungicides were added to summer oil, the addition led to no aggravation of the oil damage. Without the addition of oil, neither tetramethylthiuram disulphide nor ferric dimethyldithiocarbamate showed any control of red spider, but the infestation was checked on the corresponding trees sprayed with lime sulphur.

Trials in 1946 using phenyl mercury chloride preparations.

Plot 23.—Mercurated arsenate at 2 lb. per 100 gallons was compared with the standard lime sulphur programme in a large scale trial covering the 4-acre block of Worcester Pearmain. High winds and rain interfered with the first pre-blossom spraying, which was not completed as planned, and further rain made it necessary to defer the post-blossom application until July. Table II records the spray applications given, the mean percentage leaf area scabbed in early August and the percentage numbers of scabbed fruit at harvesting. The percentages refer to Worcester Pearmain only, and the estimates of leaf Scab infection were made from 12 trees in each 4-row plot. All fruits showing any Scab were recorded as scabbed, without any differentiation of degree of infection.

TABLE II.

Records from 1946 apple Scab control trial.

Row No.	Treatment, and stage.	Mean % leaf area scabbed.	% fruits scabbed.
1-4	3% lime sulphur, green cluster; ,, ,, pink; 1% ,, ,, fruit	0·42	23
5-8	2% merc. arsenate, pink; ,, ,, fruit	0·57	66
9-12	3% lime sulphur, pink; 1% ,, ,, fruit	0·76	68
13-16	3% lime sulphur, pink; 1% ,, ,, fruit	1·16	72
17-20	2% merc. arsenate, green cluster; ,, ,, pink; ,, ,, fruit	1·18	61
21-23	3% lime sulphur, pink; 1% ,, ,, fruit	0·90	59
General mean for lime sulphur treatments		0·81	55·5 ±6·5
General mean for mercurated arsenate treatments		0·88	63·5 ±6·3

The green cluster sprays were applied on April 18th ; the pink on April 29th ; and the fruit sprays on July 23rd.

The effect of bad timing of sprays and of the exceptionally wet summer is seen in the heavy Scab infections in all portions of the plot.

There is obviously no consistent advantage of one fungicide over the other: the difference in scabbed fruit percentages (8 per cent.) between the lime sulphur and the mercurated arsenate plots is less than half that required for significance at $P = 0{\cdot}05$. The results, therefore, indicate that the mercurated arsenate programme employed was equal to the lime sulphur programme in Scab control. No damage from the application of the mercurated arsenate occurred in this trial, but the mercurial spray was obviously without effect on red spider infestation.

Plot 17.—Plot 17 was sprayed pre-blossom and post-blossom with 2 lb. per 100 gallons of a specially compounded Venturicide containing 2½ per cent. phenyl mercuric chloride suspended in methyl cellulose solution. No records of Scab infestation were taken, but the varieties included in the trial (the Lane's in particular) were scrutinized for spray damage. No damage either to leaves or fruit was recorded in this trial.

TRIAL IN 1947 USING A PHENYL MERCURY CHLORIDE PREPARATION.

Plot 23.—The 1947 trial was intended to be a repetition of the 1946 trial with the necessary amendments. The lay-out was similar to that of 1946, but mercurated arsenate was replaced, at the same concentration, by an experimental preparation of Venturicide, consisting of 2½ per cent. phenyl mercury chloride precipitated on zinc oxide. The normal lime sulphur programme was again employed as a standard of comparison.

Two pre-blossom and two post-blossom sprays were applied, on April 25th, May 9th, May 28th and June 25th respectively. The trial, however, failed to provide results on Scab control because of the serious damage caused by the post-blossom mercurial spray. This damage took the form of leaf scorching, defoliation (approximately 60 per cent.) and almost entire shedding of crop. The few fruits remaining on the mercury-sprayed trees were severely russeted and distorted.*

As far as could be judged by casual observation both treatments gave excellent control of Scab, but it was decided that detailed recording was not justifiable in the circumstances.

TRIALS IN 1947 USING GLYOXALIDINES AND HYDROQUINONE.

Plot 23 (*sub-plot N*).—The lay-out of this trial on the block of 60 trees of Worcester Pearmain is illustrated in Fig. 2. For the pre-blossom spraying small quantities only of the undiluted heptadecylglyoxalidine (" 341 ") and hydroxyethylheptadecylglyoxalidine (" 337 ") were available. These were employed on four trees in the eastern four rows (rows 42-45) for pre- and post-blossom sprays, in comparison with four trees sprayed throughout with lime sulphur, four sprayed throughout with hydroxyquinoline and eleven left unsprayed.

The remainder of the plot received a routine pre-blossom spray of 3 per cent. lime sulphur. After blossoming, material of the compounded glyoxalidine preparations, 341 A, 341 B, and 341 C became available, and these were used in two post-blossom sprays on rows 36-41, in comparison with 1 per cent. lime sulphur, with hydroxyquinoline, and with no post-blossom spray.

In May, all trees in the plot showed a little leaf edge scorching owing to weather conditions. The scorch was accentuated by the pre-blossom lime sulphur sprays, but not by glyoxalidine or hydroquinone. In the post-blossom sprayings no phytocidal effects appeared.

Scab infection of the leaves was estimated, as previously described, on samples of approximately 250 leaves per tree, picked in mid-July. The mean percentage infection per fruit was calculated from the complete crop for each tree during the period August 14th-27th. As no significant differences were found between " 341 " and " 337 ", the figures for these products are combined and, for the same reason, the figures for 341 A, 341 B and 341 C are taken together. The results of the trial are summarized in Table III.

As indicated by the Standard Errors in Table III, there are, especially in the leaf samples, wide tree-to-tree variations within treatments. The results are, however, consistent throughout in showing that in this trial the level of Scab control secured by the glyoxalidine treatments was not significantly inferior to that obtained by the standard lime sulphur applications. This holds good for leaf counts and fruit counts both on rows 36-41 (post-blossom spraying) and on rows 42-45 (complete spray programme). Spraying with hydroxyquinoline, on the other hand, gave no significant improvements in Scab control over the unsprayed trees.

A heavy red spider infestation developed over the entire block, but was slightly checked on the trees sprayed post-blossom with lime sulphur.

Plot 23. (*Stirling Castle and Lane's Prince Albert.*)—On June 19th-21st a spray damage trial was carried out on 20 trees each of Lane's Prince Albert and

* This damage is being made the subject of a separate investigation which will be recorded elsewhere.

TABLE III.

Records from 1947 apple Scab control trial.

	Spray applications.				Mean percentage leaf area scabbed (mid-August).	Mean percentage fruit surface scabbed (end August).
	First pre-blossom, April 25th.	Second pre-blossom, May 9th.	First post-blossom, May 22nd.	Second post-blossom, June 11th.		
Rows 42-45	3% lime sulphur	3% lime sulphur	1% lime sulphur	nil	0·0650 ±0·0017	0·0750 ±0·0003
	0·001% glyoxalidine*	0·001% glyoxalidine*	0·001% glyoxalidine*	nil	0·2325 ±0·1021	0·1800 ±0·0053
	0·001% hydroxyquin.†	0·001% hydroxyquin.†	0·001% hydroxyquin.†	nil	1·0850 ±0·6421	0·4250 ±0·0110
	nil	nil	nil	nil	1·7218 ±0·9841	0·4782 ±0·0146
Rows 36-41	3% lime sulphur	nil	1% lime sulphur	1% lime sulphur	0·5866 ±0·2959	0·0351 ±0·0004
	3% lime sulphur	nil	0·001% glyoxalidine‡	0·001% glyoxalidine‡	0·1033 ±0·0057	0·0338 ±0·0006
	3% lime sulphur	nil	0·001% hydroxyquin.†	0·001% hydroxyquin.†	0·6200 ±0·0115	0·1433 ±0·0045
	3% lime sulphur	nil	nil	nil	0·9150 ±0·3202	0·0912 ±0·0007

* Glyoxalidine 341 or 337. † With hydrated lime. ‡ As Glyoxalidine 341 A, B or C.

	36	37	38	39	40	41	42	43	44	45
	Row No.									
Tree No.										N ↑
7	Gc	o	L	o	Gb	o	L	o	G*	o
6	o	Q	o	Q	o	Q	o	Q	L	Q
5	L	o	Ga	o	L	o	G	o		o
4	L	o	L	o	L	o	o	o	L	o
3	o	Gb	o	Q	o	Ga	o	Q	o	G
2	Q	o	Gc	o	Q	o	G*	o	Q	o

Planting distance .. .. 15×15 feet.

FIG. 2.

Layout of 1947 apple spraying trial at Long Ashton on Plot 23, Sub-plot N.

o No spray.	G* Hydroxyethylheptadecylglyoxalidine (337).
L Lime sulphur.	Ga Glyoxalidine preparation 341 A.
Q Hydroxyquinoline.	Gb ,, ,, 341 B.
G Heptadecylglyoxalidine (341).	Gc ,, ,, 341 C.

Stirling Castle, varieties which are specially sensitive to spray injury. The sprays used were Glyoxalidines 341 A, 341 B, and 341 C (each at a strength equivalent to 1 lb. glyoxalidine per 100 gallons) and hydroxyquinoline, also at 1 lb. per 100 gallons, plus 4 oz. of quick-lime. Each of these four treatments was applied to 4 trees of Lane's Prince Albert and 4 trees of Stirling Castle at the rate of 2½ gal. per tree. The remaining 8 trees were left unsprayed.

No damage developed on any of the trees as a result of these applications.

TRIAL AGAINST BROWN ROT OF APPLES.

TRIAL IN 1944, USING FERRIC DIMETHYLDITHIOCARBAMATE.

This trial was carried out in a plantation of large cordon trees (variety Cox's Orange Pippin) in Worcestershire, for the specific purpose of exploring the possibility of controlling codling moth, Brown Rot and red spider with a combined wash applied in late June. A description of the trial and the full results have already been published (Bennett *et al.*, 1944).

The ferric dimethyldithiocarbamate was applied as Fermate, containing 60 per cent. of fungicide, in the following mixed wash :

Fermate	2 lb.
Grade G oil emulsion ..	1 gal.
D.D.T.	1 lb.
Water to	100 gal.

Comparable plots were sprayed with copper oxychloride-lead arsenate-oil and with copper oxychloride-D.D.T.-oil.

The Fermate-oil wash left a most conspicuous black residue on the leaves and

fruit which persisted on the apples for the rest of the season as unsightly dark blotches. It caused no russeting and negligible defoliation, in contrast to the copper-containing sprays which were highly phytocidal both to leaves and fruit.

Infections by Scab on the sprayed plots were negligible and no infestation by codling moth took place. The opportunities for entry by the Brown Rot fungus were, therefore, scanty, and infection never reached epidemic proportions. The final counts of numbers of fruits showing Brown Rot and mean weight of all fruits per treatment are assembled in Table IV.

TABLE IV.

Apple Brown Rot control trial: Records of infection and fruit size.

Treatment.	Mean percentage no. of fruits with Brown Rot.	Mean weight per fruit (grm.).
Copper oxychloride-lead arsenate-oil	2·05 ±0·52	69·00 ±2·68
Copper oxychloride-D.D.T.-oil	2·15 ±0·73	62·75 ±2·93
Fermate-D.D.T.-oil	2·20 ±0·69	76·75 ±3·43
Control	10·25 ±0·92	84·75 ±3·42 (Significant difference 9·64)

While this experiment shows that the Fermate-oil spray reduced the infection of Brown Rot by four-fifths without causing injury (other than skin disfigurement), it must be emphasized that the attack was a light one. Subsequent experience in other localities has shown that Fermate spraying will not control a heavy Brown Rot infection of apples in this country (Moore, 1946).

PRELIMINARY TRIAL AGAINST BROWN ROT OF PLUMS.

These experiments were concerned only with the inhibition of spore formation on overwintered plums by spraying the mummied fruits on the tree during the winter. Trials in the laboratory indicated that the dithiocarbamate materials and the glyoxalidines were less promising for this purpose than phenyl mercury chloride. This was, therefore, used in December, 1946, for a preliminary field trial in comparison with a 0·8 per cent. dinitrocresol spray. In March, 1947, material of hydroxyquinoline became available and was also included in the experiment.

The materials were compounded for spraying as follows:

(i)	Phenyl mercury chloride	3 grm.
	66 per cent. Grade E oil emulsion (with sulphite lye) ..	75 c.c.
	Water	1 litre.
(ii)	Dinitro-*ortho*-cresol (90 per cent. pure)	9 grm.
	Gypsum (second rock grade)	9 grm.
	Estol H	1 c.c.
	Water	1 litre.
(iii)	Hydroxyquinoline sulphate	20 grm.
	Water	1 litre.

Spraying was carried out on clusters of mummied fruits occurring on plum trees of the variety Avon Cross in the Long Ashton plantations. The treated fruits were left on the trees until June 2nd when they were brought into the laboratory and kept in moist chambers to determine their ability to produce sporing pustules of the Brown Rot fungus (*Monilia fructigena*). A summary of the results obtained is given in Table V.

TABLE V.

Records from mummied plums: June, 1947.

Treatment.	Date of treatment.	Total plums.	No. showing sporing pustules of Monilia.
Dinitro-cresol	13.12.46	48	44
Phenyl mercury chloride	30.12.46	36	3
Hydroxyquinoline	26.3.47	28	24

No damage to the fruit buds was recorded.

Although the experiment was on such a small scale, the relative success of the mercury spray was noteworthy. More extensive trials on winter treatments of mummied fruits are in progress.

TRIALS AGAINST BLACK CURRANT LEAF SPOT.

The trials described below were all carried out on the block of 224 Baldwin black currant bushes set out in Plot 3 at Long Ashton in 1940. The spray treatments applied in any one year were replicated at least four times in randomized blocks. While the primary object of the trials was to use the control of defoliation (caused by *Pseudopeziza ribis*) as one indication of fungicidal performance in the field, the opportunity was taken to investigate the action of the various spray residues on the fruit after processing. The results of the 1944 and 1945 trials have already been published (Marsh and Dickinson, 1944; Adam *et al.*, 1945).

1944 trial.—This trial established the fungicidal value of Fermate in comparison with that of the standard Bordeaux spray. The mean percentage foliage retentions recorded on September 26th, 1944, were as under:

Following 2 : 100 Fermate spray on July 7th	70
,, 4 : 10 : 100 Bordeaux spray on July 27th ..	61
Control	23

1945 trial.—In 1945 a further comparison was made between the two dithiocarbamate materials. The mean percentage foliage retentions recorded on September 28th, 1944, were:

Ferric dimethyldithiocarbamate (2 : 100)	55
Tetramethylthiuram disulphide (2 : 100)	23
Control	14

In this trial, ferric dimethyldithiocarbamate was highly significantly better than the thiuramdisulphide and the latter was not better than the control.

1946 trial.—In 1946, the comparison with the disulphide was repeated and a wider range of materials was included in the trial. All spraying was done in the first week of June, and the foliage retention counts (Table VI) were made on September 17th.

TABLE VI.

Mean percentage foliage retention on black currants: September 17th, 1946.

Spray treatment.	Concentration per 100 gallons.	% foliage retention.
Zinc dimethyldithiocarbamate	2 lb.	81
Tetramethylthiuram disulphide (46% pure)	4 lb.	75
Ferric dimethyldithiocarbamate	2 lb.	71
Tetrachlorbenzoquinone (Spergon)	2 lb.	57
Folosan DB 905 (5% tetrachlornitrobenzene) ..	2 lb.	57
Salicylanilide (Shirlan AG)	2 lb.	55
Venturicide (2·5% phenyl mercury chloride) ..	1 lb.	56
Control	—	60

Statistical analysis of the data showed that the results from tetramethylthiuram disulphide and ferric dimethyldithiocarbamate were not significantly different but were inferior to those given by zinc dimethyldithiocarbamate. The remaining substances tested gave no control of black currant Leaf Spot at the concentration employed.

1947 trial.—In the 1947 trial, zinc dimethyldithiocarbamate and tetramethylthiuram disulphide were again included (at reduced strength), together with Dithane, glyoxalidine, Phygon and hydroxyquinoline. The Dithane application was made on July 4th; the remainder on June 23rd. Leaf Spot developed unusually early and the counts were made on August 6th, giving the results shown in Table VII.

TABLE VII.

Mean percentage foliage retention on black currants: August 6th, 1947.

Spray treatment.	Concentration per 100 gallons.	% foliage retention.
Dithane (25% disodium ethylene bisdithiocarbamate)	4 pt.	65
Heptadecylglyoxalidine	1 lb.	63
Phygon (98% dichlornaphthoquinone)	1 lb.	61
Zinc dimethyldithiocarbamate	1 lb.	53
Tetramethylthiuram disulphide (46% pure)	2 lb.	48
Hydroxyquinoline sulphate	1 lb.	20
Control	—	26

The 1947 trial showed that Phygon at 0·001 per cent., glyoxalidine at 0·001 per cent. and Dithane at 0·5 per cent. (=0·125 per cent. disodium ethylene bisdithiocarbamate) gave equal control of the disease: tetramethylthiuram disulphide and

zinc dimethyldithiocarbamate, both at 0·001 per cent., were somewhat less effective, while the hydroxyquinoline at this strength entirely failed to give any control.

Processing trials.—The results of the storage tests of canned fruits carrying spray residues of the materials used in 1945 have previously been published (Adam *et al.*, 1945). Briefly it was found that a deposit of ferric dimethyldithiocarbamate was not deleterious to the flavour or storage life of processed black currants, provided that the sprayed currants were first heated in an open vessel to decompose the thiocarbamate. Such heating normally occurs in the preparation of black currant syrup, purée or jam. If, however, the currants were canned as whole fruit in syrup, without previous heating, the spray residue accelerated corrosion of the can and adversely affected the flavour and appearance of the fruit.

Details of the processing trials on the 1946 crop will be given elsewhere. The storage experiment corroborated the results of the previous year and showed that tetramethylthiuram disulphide and zinc dimethyldithiocarbamate gave the same results as the ferric salt. The remaining materials tested, namely tetrachlorbenzoquinone, tetrachlornitrobenzene, salicylanilide and phenyl mercury chloride had no effect on the corrosivity of the canned fruit.

Processing trials on the 1947 crop are not yet complete.

Spray Damage Trial on Gooseberries.

This experiment was made on June 16th, 1947, using gooseberry bushes of the variety Leveller, which is outstandingly susceptible to spray damage. The following treatments were applied to the respective bushes :

(*a*) Phenyl mercuric chloride, applied as Venturicide 1 lb. per 100 gallons ;
(*b*) 8-Hydroxyquinoline sulphate 1 lb. per 100 gallons, with 4 oz. hydrated lime ;
(*c*) Dichloronaphthoquinone 1 lb. per 100 gallons, with 4 pints sulphite lye ;
(*d*) Heptadecylglyoxalidine 1 lb. per 100 gallons ;
(*e*) Flowers of sulphur, applied as a dust.

On June 30th, 1947, the bush treated with sulphur showed 76 per cent. defoliation : no defoliation was caused by any of the other treatments. The safety of heptadecylglyoxalidine and of hydroxyquinoline on the sulphur-sensitive apples Lane's Prince Albert and Stirling Castle is thus corroborated by this experiment on Leveller gooseberries.

DISCUSSION.

Apple sprays.

Among the fungicides now in commercial use on fruit crops in this country, the pre-eminent place is taken by lime sulphur. In the control of apple Scab it is the standard fungicide against which possible substitutes must be judged.

Lime sulphur is a cheap, stable, effective and tenacious fungicide, readily diluted to any concentration required. Its disadvantages are that it is somewhat objectionable to the operator (particularly when wash is blown into the eyes) ; it commonly causes some scorching of apple foliage in the spring, and it may induce leaf and fruit drop on a number of varieties in the summer. A mixture of lime sulphur and mineral oil cannot be applied to foliage and fruit without risk, and the combination of lime sulphur with lead arsenate has a limited range of safety. The concentrate is relatively

bulky, 40 lb. being required for 100 gallons of wash at 3 per cent. strength, and, being a corrosive fluid, it has to be transported in expensive steel drums. It is normally readily available, but in 1947 supplies were restricted.

An outstanding merit of lime sulphur is that it is not only a fungicide, but also an acaricide. If applied thoroughly, in conjunction with a wetter, to apple trees at the petal fall stage, it gives a measure of control of red spider.

All the solid materials tested in the course of this investigation were employed at 1 or 2 lb. per 100 gallons, a much lower concentration than that used for lime sulphur sprays. These materials consequently possess advantages in storage and transport, but in spite of the lower concentrations required, it is likely that they would prove costly to use at present.

The dithiocarbamates are not irritating to the skin and, in the trials recorded above, they have caused no damage to leaves or fruit, even when used on the sulphur-sensitive Lane's Prince Albert apple. Ferric dimethyldithiocarbamate (Fermate) has the disadvantage of leaving a black spray residue which is disfiguring and persistent. This alone would seriously limit its usefulness in apple spraying. Both ferric dimethyldithiocarbamate and tetramethylthiuram disulphide were apparently compatible with the Grade G oil sprays used on apples in the 1944 and 1945 trials. Neither of these fungicides, however, used at the recommended strengths, appears to be as effective as standard lime sulphur sprays in controlling Scab. Tetramethylthiuram disulphide has previously been recorded as giving erratic results, probably because of its chemical instability. Montgomery and Shaw (1943) have shown that this compound exhibits the phenomenon of inversion of toxicity, i.e. within a certain range, the toxicity to *Venturia inaequalis* spores decreases as the concentration of the fungicide increases.

In considering the recommendations given for the use of thiocarbamate sprays in U.S.A. and on the continent of Europe it has to be borne in mind that in these countries there is a succession of codling moth broods during the summer and, therefore, apples must be given a series of arsenical spray applications from June onwards. Any fungicide used is added to the codling control sprays and is, in consequence, frequently repeated in washes containing an arsenical. As the arsenical has the effect of enhancing both the fungicidal power and the tenacity of the spray mixture, a relatively weak fungicide is often sufficient in these circumstances to maintain control of apple Scab. It could not be expected that the same material would give equivalent results under English conditions where summer applications are fewer, and spraying with arsenicals after June is very unusual.

The sprays based on phenyl mercury chloride are of the same order of effectiveness in apple Scab control as standard lime sulphur sprays. Although the concentrate is highly poisonous, the diluted wash is non-objectionable to the operator. The mixture of phenyl mercury chloride with lead arsenate to form the material known as mercurated arsenate gives a combination of protective fungicide and insecticidal stomach poison which has been widely used in the British Isles and has proved generally non-phytocidal. The evidence on the phytocidal action of other compounded products of phenyl mercury chloride remains conflicting. Phenyl mercury chloride has no effect on red spider.

Hydroxyquinoline sulphate, as used in the Long Ashton trials, showed no fungicidal value whatsoever. It was non-phytocidal, not objectionable to the operator and devoid of acaricidal properties.

The glyoxalidine spray has only recently become available for test in this country, hence any estimate of its value must be tentative. As far as can be judged from a limited trial during a dry summer, it is as effective at 1 lb. per 100 gallons as the standard lime sulphur spray programme, and is without any phytocidal action even on sulphur-sensitive varieties. Prolonged close proximity to the concentrate (e.g. while grinding the material in a mortar) causes some irritation to the eyes, but when used in the ordinary processes of mixing and spraying, glyoxalidine has no objectionable properties. The American evidence shows that it can safely be mixed with lead arsenate and with oil, but parallel compatibility tests have not yet been made in England. As with all the other materials tested, glyoxalidine has no effect on red spider.

It would thus appear likely that where the replacement of lime sulphur for the control of apple Scab is desirable, satisfactory materials for this purpose are in sight. Suitable preparations of glyoxalidine derivatives show promise of maintaining control without risk of causing spray damage. No data are available on the likelihood of glyoxalidine spray preparations becoming available in this country, or on the cost of this fungicide, but the effective concentration of the active material is much lower than that of lime sulphur. If, however, a combined fungicidal and acaricidal action is needed no one substance has been found which will replace lime sulphur. Further investigations are necessary to determine whether the combination of a glyoxalidine derivative with white oil or with a specific acaricide will provide a practical alternative to the petal-fall lime sulphur spray on apples.

Black currant sprays.

The experiments on the control of black currant Leaf Spot confirm the view that *Pseudopeziza ribis* is, in general, more sensitive to fungicides than the apple Scab fungus. The materials of the thiocarbamate group (ferric and zinc dimethyldithiocarbamates and disodium ethylene bisdithiocarbamate) gave effective control of Leaf Spot at concentrations of 2 lb. per 100 gallons. Tetramethylthiuram disulphide has again shown erratic results, being successful in 1946, but not in 1945.

The objection to the presence of copper spray residues on black currants intended for processing is that copper, even in minute amounts, catalyses the oxidation of ascorbic acid. This is obviously a matter of importance in the manufacture of black currant products of guaranteed vitamin C content. It has been shown (Adam *et al.*, 1945) that the thiocarbamates are free from this objection, as stored canned black currants carrying thiocarbamate spray residues retain their ascorbic acid content. The disadvantage of thiocarbamate spray residues is that they may act on the tin-plate to form tin sulphide, and this reaction is accompanied by deleterious effects on the flavour and appearance of the fruit. This problem does not arise when currants are pulped before being canned (as in the production of purée or syrup), since the heat applied during pulping decomposes the thiocarbamate residue. Thiocarbamate sprays may therefore be employed on currants intended for all purposes in which the fruits are first heated whilst exposed to air. However, if the currants are to be canned as whole fruits, without previous heating, the presence of the thiocarbamate spray residues is objectionable.

Heptadecylglyoxalidine and dichloronaphthoquinone, in their first season's trial, also gave satisfactory field control of black currant Leaf Spot, but the effect of these materials on the processed fruit has not yet been determined.

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SUMMARY.

1. The assessment of field performance of fungicides at Long Ashton in 1944-47, using control of apple Scab, *Venturia inaequalis*, as the criterion, has yielded the following results :

Ferric dimethyldithiocarbamate and tetramethylthiuram disulphide are non-phytocidal, but they are inferior in fungicidal performance to lime sulphur. The spray residue of ferric dimethyldithiocarbamate on apples is disfiguring and persistent.

Phenyl mercury chloride, at the strength tested, is approximately equal to standard lime sulphur applications in fungicidal effect. The material has proved generally safe, but certain experimental preparations have caused spray damage.

Hydroxyquinoline sulphate showed no fungicidal value in these experiments.

In one season's trials, heptadecylglyoxalidine equalled lime sulphur in apple Scab control and caused no damage to sulphur-sensitive varieties.

2. In tests against Brown Rot of fruits, promising results in the inhibition of the sporing of *Monilia fructigena* on mummied plums were obtained in a preliminary winter trial of phenyl mercury chloride compounded in an oil emulsion. Spraying of apples in late June with ferric dimethyldithiocarbamate reduced the spread of Brown Rot infection when the attack was light.

3. Field tests of fungicides against black currant Leaf Spot (caused by *Pseudopeziza ribis*) show that heptadecylglyoxalidine, dichlornaphthoquinone and the dithiocarbamate sprays all provide satisfactory control of the disease. Thiocarbamate spray residues are not deleterious to canned black currants if the sprayed fruits are heated before canning, but the undecomposed thiocarbamates are objectionable in stored canned products.

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ANATOMICAL STUDIES OF STEMS AND ROOTS OF HARDY FRUIT TREES

IV. THE ROOT STRUCTURE OF SOME NEW CLONAL APPLE ROOTSTOCKS BUDDED WITH COX'S ORANGE PIPPIN

By A. BERYL BEAKBANE and ELEANOR C. THOMPSON

FULL pomological descriptions of the families of apple rootstocks used for the present study have been published by Tydeman (1933 and 1943). Briefly their history is as follows: the families were bred in 1929 at East Malling from selections of the Malling clonal rootstocks. All nineteen families had one common parent, Jaune de Metz (Malling IX), while nearly all the remaining sixteen original Malling clonal apple rootstocks were included among the other parents. Approximately one thousand seedlings were grown in nursery rows as "stools" for four years, the method of propagation being similar to that described by Knight *et al.* (1928). Wide variations were observed in the ease with which the seedlings produced adventitious roots from the stool shoots. Thirty-eight individuals were chosen from the thousand seedlings on the basis of their behaviour in the nursery, the main characteristic used for selection being the production of adventitious roots. A set of these rootstocks with nine controls from the Malling series was planted in nursery rows at 1½ ft. by 4 ft. and budded during the summer of 1939 with Cox's Orange Pippin. The trees received routine spraying and cultural treatments, but no pruning. Records of shoot growth and of the number of fruit buds and fruits were made. When the scions were three years old, four trees on each of the thirty-eight rootstocks and four trees on each of the nine Malling rootstocks included as controls were chosen for anatomical study.

The following measurements were made:

(1) Per cent. bark on seven sizes of root varying from 3 to 10 mm. diameter.

(2) Relative areas of fibres, vessels, parenchyma and rays in transverse sections of the wood of roots of 7 to 8 mm. diameter.

(3) Relative area of fibres in the bark of transverse sections of roots of 7 to 8 mm. diameter.

The per cent. bark was measured on transverse sections of the roots with an eyepiece micrometer. The percentage area of the various tissues of the wood was estimated by means of weighings of the corresponding parts cut from photographic prints of the transverse sections. A detailed description of these two methods and of the method of estimating the percentage area of fibres in the bark was given in an earlier paper (Beakbane, 1941).

In 1943, trees on eighteen of the most promising of these thirty-eight rootstocks were transplanted to a field trial, together with control trees on some of the Malling clonal rootstocks. The remaining trees in the nursery trial were destroyed. Data on the growth and fruiting of the transplanted trees (hereafter referred to as the field trial series) were examined in relation to the information already gained by an anatomical investigation of the structure of the roots of the four trees on each rootstock in the nursery trial.

1. PER CENT. ROOT BARK.

The per cent. bark in roots of 7 to 8 mm. diameter varied from 43 to 73 in the new clonal rootstocks (see Plate I) and from 40 to 67 in the nine Malling rootstocks included as controls (see Plate II).

The one-year-old trees bore no fruit buds, but during the second and third years most of the trees on rootstocks with a per cent. root bark of over 60 bore a moderate number of fruit buds, while only two rootstocks with a per cent. bark below 50 bore any fruit buds at all (see column 9 of the Table). The fruitfulness of the field trial series four years later is indicated by the number of fruit buds (see column 10 of the Table). A comparison of columns 9 and 10 with column 1 shows that trees with a high per cent. root bark have more fruit buds than those with a low amount of root bark thus indicating a relationship between per cent. root bark and fruit bud production.

While there was no relationship between per cent. root bark and vegetative growth of the three-year-old Cox scions in the nursery trial, there were indications of a fair correlation between the per cent. bark and growth in the field trial series at seven years old (column 11).

The vegetative vigour of nine-year-old trees on four of the Malling rootstocks is given in column 12. These data have been obtained from the mean vigour of sets of Cox's Orange Pippin trees grown on the five different soil series described by Rogers (1946). The close correlation between per cent. bark and vigour in these trees strengthens the evidence in favour of the deductions drawn from the seven-year-old trees. It appears, therefore, that the per cent. bark of roots from three-year-old trees may be used to forecast, with a fair measure of accuracy, the ultimate vigour which a rootstock will impart to a scion variety.

The analysis of the data for per cent. root bark on seven sizes of root indicates that a relationship exists between root size and per cent. bark. This relationship differed in different rootstocks in that the per cent. bark decreased more rapidly with increasing size of root in the vigorous rootstocks than in the dwarfing ones. (Fig. 1.)

2. RELATIVE AREA OF FIBRES, VESSELS, PARENCHYMA AND RAYS IN TRANSVERSE SECTIONS OF THE WOOD OF ROOTS OF 7 TO 8 MM. DIAMETER.

A summary of the data on percentage area of fibres, vessels, parenchyma and rays in transverse sections of the root wood is given in columns 2, 3, 4 and 5 of the Table. In the roots of the new clonal rootstocks the percentage area of fibres varied from 17 to 35, of vessels from 7 to 21, of parenchyma from 24 to 41 and of rays from 14 to 44. From these data the percentage of living tissue in the wood of the roots has been calculated for each rootstock and is given in column 6. In general, the rootstocks which induced dwarf and fruitful trees at seven years old were those with a relatively small amount of fibres and a large amount of rays in their roots. There appeared to be a slight tendency for the area of vessels, as shown in transverse section, to be related to the growth and fruiting of the scion. Vessel size, as distinct from area, was not measured in the roots of this set of rootstocks. Earlier work has, however, given indications that the more vigorous rootstocks possess larger vessels in their roots than the dwarfing ones (Beakbane and Thompson, 1939; and Beakbane, 1941).

TABLE.

Comparison between root structure, growth and number of fruit buds in Cox's Orange Pippin on some new clonal apple rootstocks and nine Malling rootstocks.

Column	1	2	3	4	5	6	7	8	9	10	11	12
		Percentage area of elements in the wood of the root.										
Rootstock number.	Percentage root bark at 7-8 mm. diameter.	Fibres.	Vessels.	Parenchyma.	Rays.	Percentage living tissue in the wood.	Percentage area of fibres in the bark.	Percentage living tissue in the whole root (wood and bark).	Total number of fruit buds, 1940-1942.	Number of fruit buds per metre wood growth at 7 years old.	Amount of shoot growth in decimetres at 7 years old.	Cross-sectional area (sq. cm.) of trunk at 9 years old.
XIII	40	32	15	35	18	53	5·67	69	0	—	—	—
XII	40	35	13	34	17	52	4·18	69	0	0·1	198	—
XVI	40	32	13	38	16	55	4·13	71	0	—	—	91
3438	43	31	9	37	22	60	3·12	75	0	0·7	108	—
V	44	26	10	37	27	64	1·93	79	0	—	—	—
XV	44	39	13	29	18	48	2·80	70	0	—	—	—
I	45	29	11	42	18	59	3·40	76	0	—	—	—
349	46	35	11	33	21	54	2·92	74	4	—	—	—
342	46	33	10	36	20	57	2·17	76	0	—	—	—
3434	48	32	12	36	20	56	2·10	76	0	—	—	—
345	48	30	11	35	24	59	0·75	79	0	—	—	—
343	48	34	10	33	23	56	1·43	76	2	—	—	—
344	49	26	13	39	21	61	0·82	80	0	—	—	—
348	51	35	10	32	22	55	2·03	77	14	—	—	—
II	51	28	11	42	19	61	3·59	79	13	10·3	91	66
3414	52	24	13	41	21	63	1·05	82	17	7·6	95	—
347	52	34	11	34	20	54	0·52	78	1	—	—	—
3425	52	26	12	34	28	62	1·32	81	0	—	—	—
3430	53	29	21	35	14	50	4·82	74	0	0·5	286	—
3429	54	33	8	34	24	58	3·12	79	0	—	—	—
3428	54	34	13	32	21	53	2·58	77	52	10·6	75	—
3433	54	24	10	31	35	66	0·48	84	12	—	—	—
3432	55	24	13	25	38	63	0·38	83	3	11·6	61	—
341	55	29	13	34	25	59	5·15	79	11	13·6	86	—
3422	57	17	12	31	40	71	0·75	87	1	11·2	69	—
3436	57	28	8	29	34	64	0·73	84	10	11·8	69	—
VII	59	32	11	32	25	57	4·65	80	3	10·1	71	55
3437	60	19	12	34	34	69	0·47	87	14	16·8	35	—
3435	61	22	11	27	40	67	0·45	87	43	9·1	80	—
3424	61	21	7	38	34	72	0·28	89	36	14·2	37	—
3417	62	21	8	32	39	71	0·20	89	0	—	—	—
3420	62	18	13	25	44	69	0·18	88	13	16·7	33	—
3421	63	20	9	32	38	71	0·22	89	31	22·3	41	—
3427	63	21	15	31	32	64	1·92	85	2	—	—	—
3419	63	21	11	36	33	68	0·50	88	68	16·6	28	—
3416	63	22	9	39	29	69	0·07	88	32	—	—	—
3418	64	28	8	39	24	64	1·40	86	15	—	—	—
3413	64	19	10	37	34	71	0·02	89	50	23·5	21	—
3412	65	20	13	29	38	67	0·40	88	14	—	—	—
IX	67	20	9	35	36	71	0·03	90	22	14·7	53	26
3410	67	19	15	34	32	66	0·68	88	23	—	—	—
3411	67	21	12	33	34	67	0·18	89	16	—	—	—
346	69	22	10	35	33	68	0·08	90	—	—	—	—
3423	70	21	11	32	36	68	0·05	90	30	16·8	41	—
3431	72	21	9	31	38	69	0·13	91	44	22·9	19	—
3426	72	25	12	24	38	62	0·10	90	26	31·8	10	—
3415	73	17	9	37	38	75	0·22	93	43	—	—	—

The percentage of living tissue in the wood of the roots of the new clonal rootstocks varied from 50 to 75. While at both ends of the range there appeared to be a correlation between per cent. bark and the amount of living tissue in the wood, those rootstocks in the middle of the range, with 50 to 60 per cent. root bark (i.e. a medium thickness), possessed wood structures with a range of living tissue varying from 50 to 71 per cent. It is probable that from rootstocks such as these, individuals

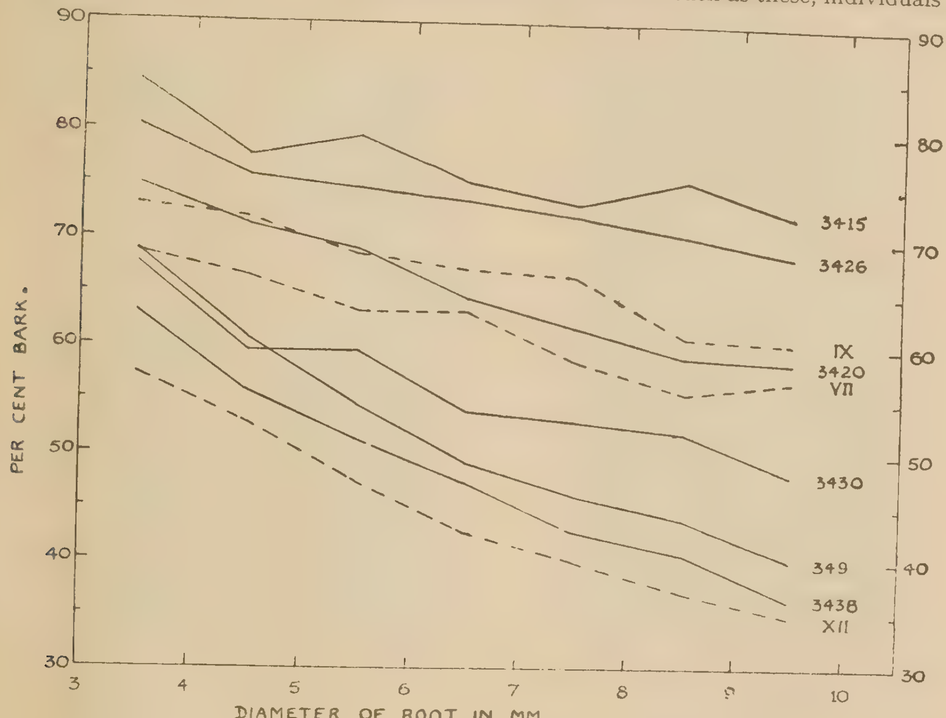


Fig. 1.
Diagram showing relation between per cent. bark and root size.

could be selected which might induce vigorous growth in the early years, combined with heavy cropping after about six or seven years. The structure of the roots of Malling VII, a rootstock known to behave in this way, falls within these limits. On the whole the range of variation in the amount of the four kinds of tissue elements composing the wood was similar in the new clonal rootstocks to that in the nine Malling rootstocks. The vessel area of the new rootstock No. 3430 was outstanding, and was greater than that of the most vigorous of the Malling clonal rootstocks. The possibility that this rootstock may have a greater number of chromosomes than the rest of the series is being investigated.

3. Relative area of fibres in the bark of roots of 7 to 8 mm. diameter.

In the roots of the new clonal rootstocks, the percentage area of fibres in transverse sections of the bark varied from 0·02 to 5·15, with a tendency for those with

a lower percentage of living tissue in the whole root to have a greater relative area of fibres in the bark (see column 7).

4. Percentage area of living tissue in the wood and bark together.

From the measurements of per cent. bark and of per cent. living tissue in the wood and bark separately, the percentage area of living tissue in the root as a whole was calculated and found to vary from 69 to 93 and to be correlated with vegetative vigour and fruitfulness. Most of the trees on rootstocks with a percentage of 80 or less of living tissue in the root had few or no fruit buds during the first three years, while, with few exceptions, those with 85 per cent. or more of living tissue in the root had a moderate number of fruit buds over the same period (columns 8 and 9 of the Table). Rootstocks with a high proportion of living tissue in the roots were less vigorous than those with a small amount (see columns 8 and 11 of the Table).

DISCUSSION.

One of the main deductions to be made from the data presented on the structure of the roots of this series of rootstocks appears to be that in apples, the per cent. root bark is the best single criterion to adopt when attempting to forecast the age of bearing and ultimate vigour of trees on a series of new rootstocks. The addition of further information on the wood structure of the root would add to the chances of accurate prediction of the behaviour of the tree on a given rootstock, since there is some evidence for supposing that the percentage area of wood fibres and wood rays in the roots of the rootstock is related to the vegetative vigour and fruitfulness of the scion variety grown on it.

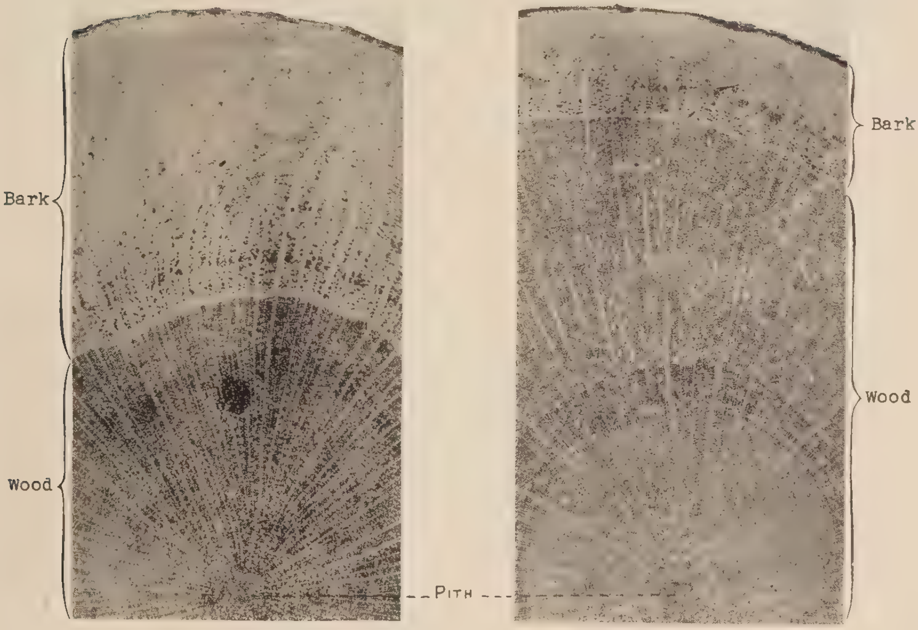
The structure of the roots of some of the rootstocks of intermediate vigour is of interest since it may partly explain the influence of rootstocks such as Malling VII, which induce intermediate vegetative vigour combined with a relatively precocious fruiting habit in scions grafted on them. In such trees the amount of root bark approaches that of the dwarfing rootstocks while the wood structure of the root resembles that of the vigorous rootstocks. Since it has been shown that rootstock differences in per cent. root bark increase with increasing root size, it follows that the full effect of such differences would not become apparent until the tree was several years old and that, in young trees, the wood structure of the roots of the rootstock might have a proportionately greater influence on growth than in later years. Therefore, it seems reasonable to suppose that the root structure of a rootstock such as Malling VII may, to some extent, account for the influence which it has on a scion variety, namely, that of inducing vigorous growth in the early years, combined with comparative precocity in fruiting.

SUMMARY.

1. An account is given of the structure of the roots of thirty-eight new clonal apple rootstocks and of nine Malling rootstocks used as controls.

2. Data on the percentage of bark in roots of a given size indicated that there was a correlation between this percentage in the roots produced by a given rootstock and the vigour and precocity of the scion grafted on the rootstock. Further data

PLATE I.



Rootstock No. 3415 (dwarfing).
(73 per cent. bark).

Rootstock No. 3438 (vigorous).
(43 per cent. bark).

Photomicrographs of parts of transverse sections of the roots of two new clonal apple rootstocks showing the width of the bark. (×33)

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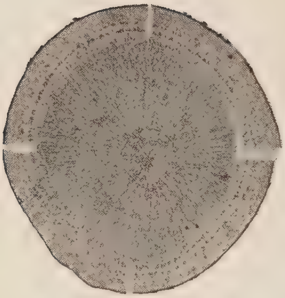
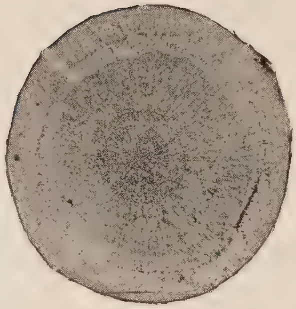
PLATE II.

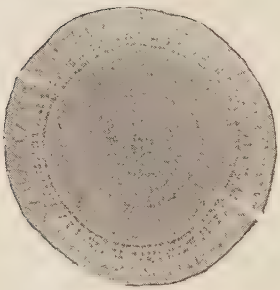


M. I (45% bark)

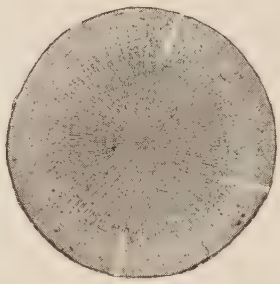


M. II (51% bark)

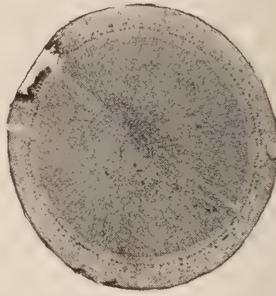
M. V (44% bark)



M. VII (59% bark)

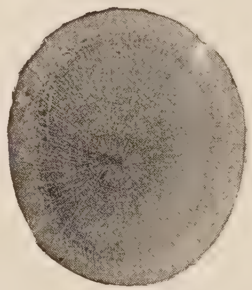


M. IX (67% bark)

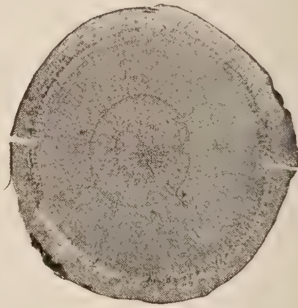


M. XII (40% bark)

M. XIII (40% bark)



M. XV (44% bark)



M. XVI (40% bark)

Transverse sections of roots (7–8 mm. diameter) of some of the Malling Clonal Rootstocks, showing width and amount of bark. (×9)

on different sizes of roots indicated that, (i) there was a relationship between root size and percentage bark, and (ii) this relationship differed with different rootstocks in that the percentage bark decreased more rapidly with increasing size of root in the vigorous than in the dwarfing rootstocks.

3. The structure of the wood of the rootstock roots, in particular the amount of fibre elements and ray cells, was shown to be related to the vigour and fruitfulness of the scion variety.

4. The percentage area of living tissue in the wood and bark of the root together was found to be related to the vigour and fruitfulness of the scion variety.

ACKNOWLEDGMENTS.

The writers are indebted to Mr. H. M. Tydeman for the trees used for the present study, and to Mr. A. P. Preston for unpublished data on the growth and fruiting of trees on a range of new clonal rootstocks.

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EXAMINATION (AFTER INJECTION WITH A DYE) OF A DOUBLE WORKED PLUM TREE SHOWING INCOMPATIBILITY

By W. A. ROACH and ELEANOR C. THOMPSON

Introduction.

An interesting case of apparent incompatibility in plum trees was observed by Montgomery, Moore and Hoblyn (1943). The trees had been raised by budding President on rooted layers of Myrobalan B in 1932 and by grafting a further scion variety (Victoria) in 1935 on each of the two or three branches formed, at a point about six inches above the crotch. In 1940 the fruit on many of these trees ripened early, the leaves were yellowish green and fell early.* By August, 1943, the trees were stunted in growth and showed every sign of incompatibility. By 1946 a few trees had died, and the condition of others had become worse, whereas trees of the composition Victoria/Victoria/Myrobalan B and Victoria/Myrobalan B/Myrobalan B seemed healthy. From previous experiments it was known that President on Myrobalan B is compatible. One of the unhealthy trees seemed of sufficient interest to merit detailed examination, because it was more severely affected on one of its sides than on the other. The general features of the tree are shown diagrammatically below. The possibility of resistance to movement of liquids across the two types of union (i.e. Victoria/President and President/Myrobalan B) was tested by the injection of a dye solution followed by an examination of their structure.

Injection.

Eight holes were bored alternately above and below each union and so placed as to divide the circumference into equal parts. These holes were kept full of a 1 per cent. aqueous solution of water-blue until a cautious removal of bark showed that the dye had crossed the union. One hole was also bored half-way up the main trunk and treated similarly.

Excavation and examination of roots.

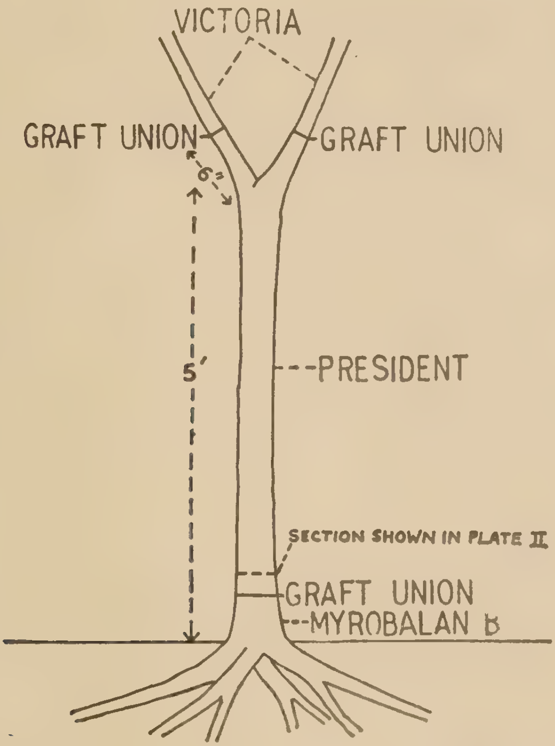
A detailed excavation of the root system by the skeleton method (Rogers, 1928) was made by Messrs. D. M. Hacking and A. P. Preston to determine whether any abnormality was visible in the roots. Most of the roots appeared normal. They extended well beyond the spread of the branches and penetrated to a depth of 1·4 metres. But a few dead roots were observed. Some of these died as a result of mechanical damage in cultivation, but there were also a few occurring below cultivation level for which there was no obvious cause of death. All the latter were found to be in direct connection with the unhealthy branch.

Distribution of dye in surface of wood.

After excavation, the bark was removed from the woody framework of the tree so that the extent of the dye movement could be observed. Browning of the wood was largely prevented by the application of a saturated solution of sulphur-dioxide in water.

* The writers are indebted to Dr. Montgomery, East Malling Research Station, for these facts.

The surface of the wood became dyed as a result of each injection. In the main trunk a coloured strip extended upwards and downwards from the injection hole. The colour decreased in intensity with increasing distance of travel. The dye had travelled equally freely in the comparatively healthy branch both upwards and downwards, and there was no evidence of any sudden decrease in intensity of colour on passing through the union. The same was true of the upward movement of dye in the unhealthy branch, but there were indications of a slight decrease in intensity



Diagrammatic representation of the double worked plum tree investigated.

of colour on crossing the union downwards. A surface examination of the union between the President stem and the Myrobalan B rootstock showed similar slight indications of a decrease in depth of colour on crossing the union in both the upward and downward directions ; but they were not sufficiently marked to lead to a definite conclusion.

Internal distribution of dye and quality of union.

Transverse cuts were made above and below each union and the resulting blocks were cut and split radially. Some cuts were made through the dye tracks. Each of the Victoria/President unions appeared to be perfect. Photographs of a

radially split surface and a radially cut surface through the President/Myrobalan union are shown in Figs. 1 and 2, Plate I. Similar slight indications of the decrease in colour, seen on the surface, extended to a few annual rings in depth (see Fig. 2, Plate I, and Fig. 5, Plate II). In the right-hand top corner of Fig. 2, Plate I, will be seen a wide band of tissue labelled F ; that was dyed blue. Most of this dye did not reach the union. But on the left of this band there is a narrow strip of dyed tissue passing through the union and continuing downwards without apparent diminution of intensity. To the left of this band there is also a much narrower one of similarly dyed tissue, H, passing through the union, the blue colour being visible from the union to a point half-way between it and the top of the picture, and to an equal distance below. Between these two blue strips will be seen a definite discontinuity, D, between the President stem and the Myrobalan rootstock ; and there is another discontinuity to the right extending to the surface of the wood.

Fig. 1, Plate I, shows one of the split pieces of wood. The split passed so cleanly through the union in the older wood from A to B (which corresponds to AB′ in Fig. 2) that it is only just possible to determine the exact position of the union in the actual specimen. In the younger wood from B to C, however (which corresponds to B′C′ in Fig. 2), the President stem tissues are separated completely from the Myrobalan rootstock tissues. But on the split surface on the opposite side of the block to the one shown in Fig. 1, there is excellent union between the President and the Myrobalan tissues over one narrow area. This is shown in Fig. 1A at E, where the President tissues, E, are continuous with those of the rootstock. A number of such small isolated strands of woody tissue between stock and scion were found, but they made up only a small fraction of the total wood of the region B-C. Probably these strands were in direct connection with the small side shoots or "feathers" that were removed from the President stem before they had attained any appreciable size ; but there is at present no proof of this. It is hoped to test this possibility when other suitable material becomes available. Although practically the whole of the " union " in the region BC broke cleanly under strain, dye passed across part of it without sudden diminution in intensity ; and this was true of places where the union could be seen with the naked eye to be structurally imperfect. Anatomical investigation by Miss Mosse showed a thin layer of living parenchyma cells between the President and Myrobalan woody tissues. This would seem to indicate that wood vessels, or tracheids, are not essential for transference of dye across the union. But it is evident that this type of parenchymatous connection is not efficient enough to maintain the tree in a healthy condition.

It is of interest to determine what change in treatment corresponded to the change from good to bad joining up at B. The annual rings could not be counted with certainty because those at the centre of the tree were obscured by the uneven browning characteristic of heartwood ; and the rings formed in the last few years of the tree's life, when growth was slow and uneven, were equally difficult to distinguish. But, as far as could be judged, the point B, at which the union ceased to be quite continuous, corresponded to the time at which the President " feathers " were removed completely from the stem, i.e. in May, 1938 (Montgomery, Moore and Hoblyn, 1943, p. 55). To test this possibility further transverse sections were cut until one was obtained passing through the centre of one of the snags left when the " feathers " were removed. A photograph of one of these is reproduced in Fig. 5, Plate II. The section was cut about an inch above the top of the radial sections

illustrated in Figs. 1 and 2, Plate I. This "feather" was cut close to the bark and therefore marks the growth ring for 1938, during which year it was cut off.

The beginning of this 1938 growth ring was followed down a radial section to another transverse section (not illustrated) cut a few millimetres above those illustrated in Figs. 3 and 4, Plate I, which show the upper (transverse) surface of the blocks of wood illustrated in Figs. 1 and 2. The beginning of the 1938 growth ring is indicated by an arrow in Figs. 3 and 4. It will be seen that the beginning of this ring corresponds with B and B′.

A further coincidence, seen quite clearly in the specimens, may just be seen in Figs. 1 and 2, Plate I. The change from dead heartwood (K) to living wood just below the union corresponded exactly with the point at which the union became unsatisfactory. Fig. 5, Plate II, also shows the extent of the heartwood. Other dark areas L and L′ extending from the periphery inwards, were those dyed blue by the injections. In those labelled L′ the dye penetrated the heartwood. The heartwood had advanced half an annual ring, or more, farther on the right of the points X and Y than on the left, i.e. into or beyond the 1940 annual growth ring, the beginning and end of which is indicated by widely-spaced dots. The lines separating the tissues in direct connection with the healthy, from those of the unhealthy branch were followed by eye from the crotch along the 5-ft. length of stem to the section shown in Fig. 5. One of these lines led exactly to X and the other to Y′. There is no reason to suppose that the division between healthy and unhealthy tissues corresponded exactly with the division between the two branches. Y and Y′ correspond sufficiently closely, therefore, to suggest that the unhealthiness of the right-hand branch, in comparison with the left-hand one, was connected with the fact that the change into dead heartwood had proceeded farther to the right of X and Y than to the left.

DISCUSSION.

The above facts seem to justify the conclusion that the union between the President intermediate stem and the Myrobolan B rootstock ceased to be entirely satisfactory when the foliage was "changed" from President to Victoria; but that the unsatisfactory nature of this union did not become apparent until the death of all the wood formed previous to this change.

This would suggest that while the tree was healthy it was dependent to an appreciable extent on the inner rings of sap-wood that were formed before the union became unsatisfactory. MacDougal, Overton and Smith (1929, p. 35; see also Fig. 20, p. 63) showed that dye solution injected into the main stem of an eleven-year-old willow tree moved from inner to outer rings as it ascended the tree. One of the writers (W.A.R.) in unpublished work has proved that the same is true for a twenty-year-old apple tree. McDougal, Overton and Smith found that in trees such as the walnut and chestnut, the wood of which becomes differentiated into sap-wood and heartwood, only the sap-wood conducted acid fuchsin solution. It is somewhat surprising that water-blue, which was chosen because it is not usually conducted as freely as acid fuchsin (Roach, 1938, p. 61; 1939, p. 214), was conducted to a limited extent in the outer layers of heartwood in the plum. It cannot be assumed, however, that since liquid injected into an inner ring of sap-wood moves into an outer one, therefore any of the inner rings are efficient conducting tissues in the normal tree; because leaves are in direct connection only with tissues in the current annual ring

and are not in direct connection with those in inner rings. Moreover, movement of injected liquid from an inner to an outer ring is much slower than its longitudinal movement within a single annual ring. It is hoped at some future date to test by means of "tracer" elements the extent to which in a normal tree the woody tissues inside the current annual ring are efficient conductors.

From the foregoing it would appear that the incompatibility found in this case was the result of the failure of the cambium to differentiate conducting tissue. The work of Brenchley and Thornton (1925) on the formation of root nodules in the pea demonstrated a close connection between a single mineral element, boron, and the differentiation of parenchyma cells into conducting tissues; and in unpublished work of one of the writers (W.A.R.), peas grafted on themselves formed perfect unions when grown in a water culture solution containing boron, but formed imperfect unions when grown in a culture solution lacking this element.

Analyses were carried out on a sample of leaves from one tree of each of the following "compositions"; Victoria/Victoria/Myrobalan B; Victoria/Myrobalan B; Victoria/President/Myrobalan B, showing no sign of incompatibility; Victoria/President/Myrobalan B bearing small leaves and making poor growth; Victoria/Utility/Myrobalan B. The samples differed so markedly in chemical composition that in the absence of a sufficient number of replicates no statement can justifiably be made, but the analysis of a comprehensive series of samples is to be undertaken during the coming season. Although the results of analyses of plum leaves did not point to a deficiency of the particular element boron, (rather the reverse) it is possible that some other abnormality in mineral composition may be associated with the incompatibility and similarly affect the differentiation of parenchyma into conducting strands.

SUMMARY.

A double worked plum tree, Victoria/President/Myrobalan B, showing signs of incompatibility in one of its two branches more than in the other, was injected with a dye solution.

The framework of the tree was sawn and split to study the distribution of the dye, the structure of the unions and the distribution of heartwood.

A visible deterioration of the union was found to correspond with the removal of all the President foliage, but the health of the tree was not affected until all woody tissues formed previous to this had become changed into heartwood, rendering the tree dependent on the later imperfectly formed part of the union. The transformation to heartwood had progressed further in those tissues in direct connection with the unhealthy branch than in those supplying the healthy one.

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PLATE I.

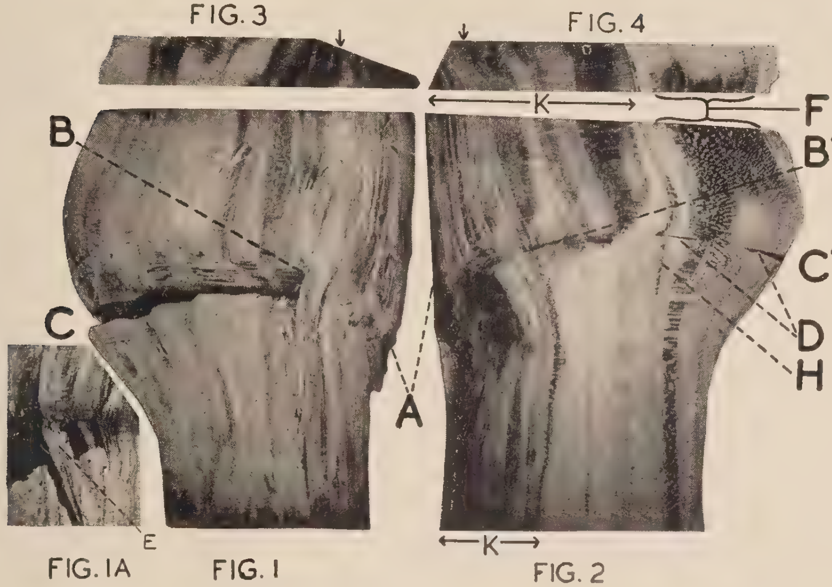

Fig. 1.

Radial longitudinal split through President/Myrobalan graft union showing satisfactory portion from A to B and incompatible portion from B to C. In this latter portion the President tissues have become almost completely detached from the Myrobalan B tissues.

Fig. 1a.

E shows a small conducting strand still directly connecting the Myrobalan B tissues with those of President, whilst surrounding it, the two tissues have become separated.

Fig. 2.

Cut and polished radial longitudinal section through same union as in Fig. 1. AB′ is the satisfactory portion of union, B′C′ an incompatible portion. D, two areas of discontinuity between President and Myrobalan tissues. F, H, tissues dyed blue by the injections. K, heartwood.

Figs. 3 and 4.

Views of transverse sections cut through tops of blocks of wood shown in Figs. 1 and 2. The arrows indicate tissues in direct connection with B or B′.

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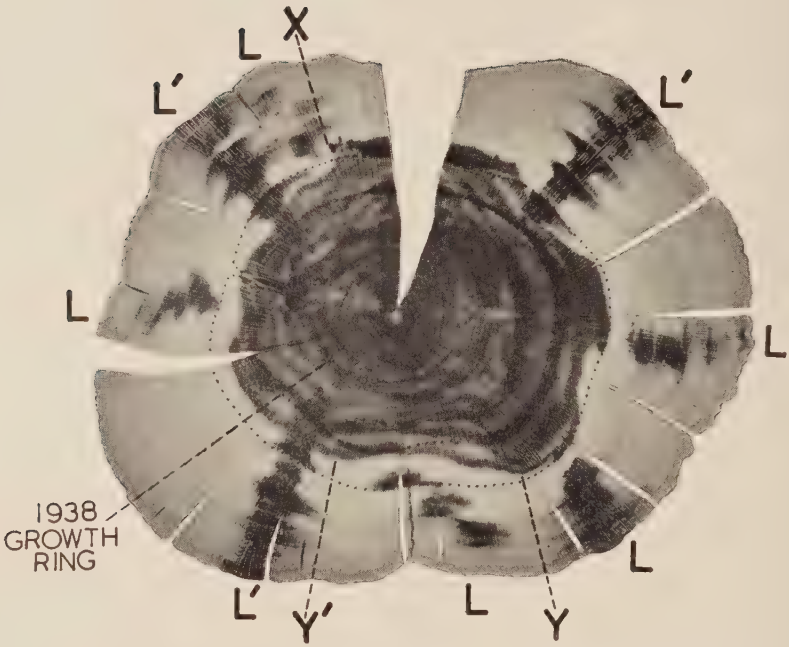

FIG. 5.

Transverse section cut about an inch above the one shown in Plate I and passing through the cut end of a small branch or "feather" known to have been removed in 1938. Dotted lines mark the beginning and end of the 1940 annual growth ring. The dead heartwood extends farther outwards to the right of XY than to the left. The junction between the healthy and the unhealthy branch was traced down the trunk to XY′, the healthy branch being on the left. L and L′, areas dyed blue by the injections. L′ shows penetration of dye through heartwood.

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6

ROOT STUDIES

XI. RASPBERRY ROOT SYSTEMS

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The study of root systems has been one of the major interests at the East Malling Research Station, where in recent years several workers—especially W. S. Rogers—have carried out many investigations on the subject. The results of this work have been published in a number of reports, and the following account forms the eleventh of the series. Among the fruit plants whose roots have been studied by excavation are apple, pear, gooseberry and black currant ; and a review of this and other work on root systems has been published (Rogers, 1939). Though many of the fundamental findings concerning the relation of certain fruit plant roots to soil profile characters can probably be applied fairly generally, each kind of fruit plant may have its own peculiarities. It is therefore desirable that the whole range of fruit plants should be studied in due course, for the importance of root environment has already been demonstrated clearly.

Information on the spread and depth of the roots of the raspberry is of special importance in connection with the planning of the best cultural practices as well as with the elimination of diseased or rogue plants from propagation beds in full confidence that no adventitious shoots or suckers (locally termed " spawn " or " colts ") will arise from roots that remain in the soil. Such information is also important in the layout of variety trials. The author had also a special interest in studying the roots of a small fruit plant, such as the raspberry, with a view to mastering a procedure which might be applied in the Argentine to root studies of the grape vine which is grown there in a somewhat similar way.

Practically no detailed studies of the raspberry root system have come to the writer's notice, though some brief notes on the subject were recently published in New Zealand by Hudson (1947) in which the variation in spread and depth in relation to shallow or deep soils was discussed.

Material, locality and method.

The material employed was growing in the raspberry variety collection at East Malling, the varieties being in single rows, spaced 6 ft. apart. The canes were originally planted 2 ft. apart in the row, and at the time of excavation they formed a more or less continuous row, with spaces of 8 ft. between varieties. They were thus growing under more or less normal commercial conditions.

The varieties chosen for study were Norfolk Giant, at present the most widely grown variety in England ; Newburgh, a variety recently imported from the U.S.A. ; and St. Walfried, another of the newer varieties. Three plants of each variety were excavated, and one of each was reconstructed later in a frame. The varieties Newburgh and St. Walfried had been planted in March, 1941, and Norfolk Giant in December of the same year. The excavations were made in November and December, 1946. The plants were thus approximately five years old when excavated.

The soil was a medium sandy loam, of the Malling Series, having some 40 cm. of brown top soil, about 25 cm. of a lighter coloured and sandier subsoil, passing into heavier material at a depth of 65 cm. ; below this there were pieces of ragstone rock interspersed amongst a sticky clay soil down to a depth of 180 cm., which was the maximum depth of the excavations. The solid rock was not reached.

The method of excavation used was that described by Rogers and Vyvyan (1928, 1934) under the designation of "Skeleton Method", in which a trench is dug outside the area overlying the roots of the plant and is gradually enlarged by strips, of 50 cm. width at a time, towards, and finally beyond, the plant. (Plate I, Figs. 5 and 6.) As the roots are encountered they are labelled, and then drawn on a plan which records their position and their depth. In this way the whole root system can be recovered practically intact, provided the work is carefully done. Later, it can be reconstructed in a frame and photographed. (See Plates II and III.)

The procedure results in the production with comparative ease of a fairly accurate picture of the whole root system, although much slow and careful work is required, especially with plants having such fragile roots as raspberries. For these the method of washing the earth away with water, as used by King (1892) and Pavlychencko (1937), might have certain advantages, and would at least eliminate the drying up of the rootlets during the work.

GENERAL CHARACTERS OF ROOT SYSTEMS.

The general characters of the raspberry roots as shown by the excavations were a relatively wide spreading system, varying from sparse to fibrous for the same plant, and including both shallow and deep roots. The older roots are woody and a number of sucker shoots arise from them at various places. These characters can be seen in Plates II, III, IV and V, and will be described more fully below.

DEPTH OF ROOTS.

In view of the common belief that the raspberry is a shallow rooted plant it is interesting to record that, in every plant excavated, some vertical roots were found descending to depths varying between 96 and 175 cm. This compares with about 150 cm. noted by Hudson in New Zealand. None of the vertical roots resembled a true tap root, which is not surprising since the plants had all been propagated vegetatively.

The roots could be classified roughly into two types, according to their direction in the soil. Some (a minority) descended vertically, and others undulated gently, that is to say the tip had grown in downward-sloping, horizontal and upward directions at different times. This undulating character is clearly revealed by depth measurements made at intervals horizontally along four typical roots (A, B, C and D) :

Root A : 20, 25, 20, 35, 59, 13, 18, 60 cm.
,, B : 18, 50, 20, 10 cm.
,, C : 28, 31, 42, 20, 12 cm.
,, D : 5, 30, 20, 11, 35 cm.

This feature is also shown in the photographs reproduced, especially in Fig. 8, Plate II; also in Fig. 6, Plate I, in which the variation in depth of a horizontally running root, and some vertically descending roots, can be seen *in situ* in the excavation trench.

TABLE I.

Percentage root weight at different depths.

Depth (cm.)	Norfolk Giant.	St. Walfried.	Newburgh.	Average.
0-25	76·8	68·3	72·2	72·4
25-50	20·6	23·1	20·2	21·3
50-75	2·1	5·5	3·3	3·6
75-100	0·5	2·6	2·3	1·8
Over 100	0·0	0·4	2·0	0·8

An attempt was made to obtain figures for the amount of roots at different depths by dividing the root systems at various levels and taking oven-dry weights. Only one plant of each variety was available for the purpose, but the results given in Table I show fair agreement with field observations. In all cases about 70 per cent. of the total root weight was found in the upper 25 cm. of soil, and a little over 20 per cent. between 25 and 50 cm. deep. This does not present a true picture of the distribution of the fine absorbing roots, however, for the thicker roots in the upper layer make up the greater part of the weight. A detailed excavation by the " Block Method " would be needed to establish the proportion of finer roots at various depths, but it can at least be said that well over 20 per cent. of the fine absorbing rootlets were deeper than 25 cm.

SPREAD OF ROOTS.

Figs. 1-3 and Plates II-III show that the spread of the root systems was much greater than that of the branches, and this corresponds with similar observations in previous investigations with other plants. The root systems were very often quite asymmetrical, and while this could be explained in some instances by competition between plants in the same row, there were cases in which no such relation could be found. Evidently other factors, such as nutrients and moisture concentration, must also affect the root distribution. In Norfolk Giant very marked root competition was evident, and this suggested that this vigorous variety requires an increased planting distance. Fig. 4 shows that in the actual planting distances in use there is ample possibility of root competition, as the whole area of soil is full of roots of different plants.

The longest roots measured 180 cm. from origin to tip, but usually the length was rarely over 100 cm. The roots seemed to follow the line of least resistance in the soil and it was quite a common occurrence to find them growing inside old decayed roots or earth-worm holes. Several roots of Newburgh growing through an old decayed plum root are seen in Fig. 11, Plate IV.

OLD AND NEW ROOTS.

The largest roots found had diameters of about 20 mm., but the normal roots were rarely more than 3 or 4 mm. in diameter. A root might vary in thickness

several times along its length, and it was quite common to find that the greatest diameter of a root was at some distance from its base. Thus there does not seem to be a direct relation between the length of raspberry roots and their diameters. Roots were commonly thicker near the points where sucker shoots had grown.

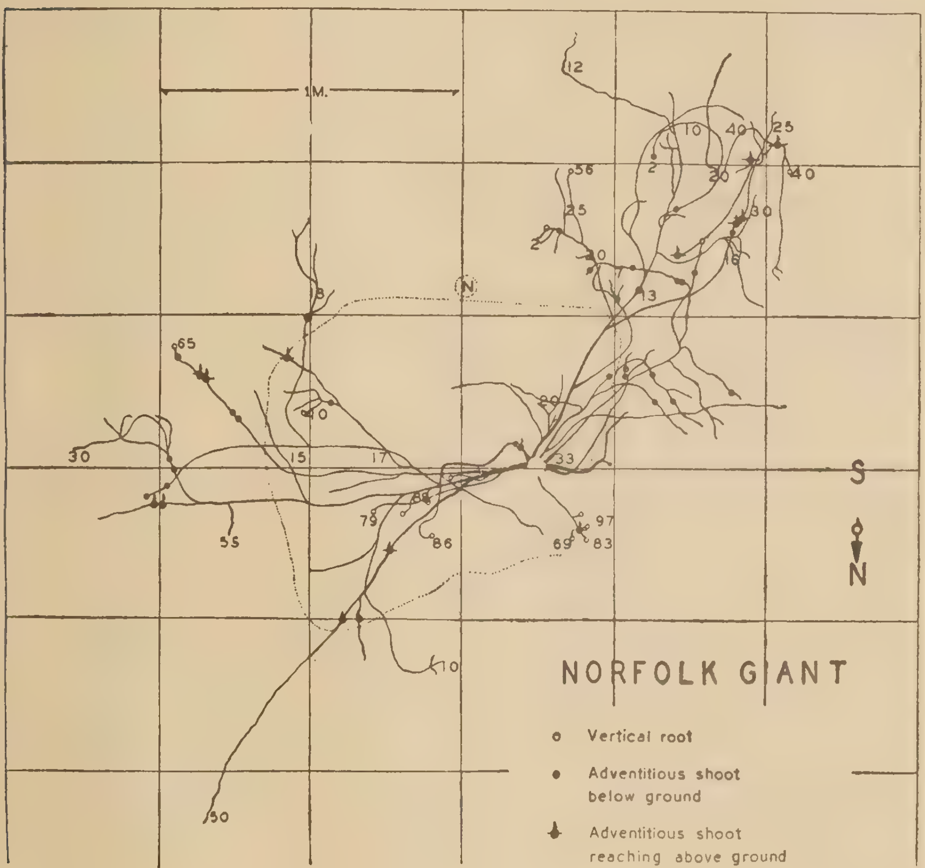

FIG. 1.

Root plan of Norfolk Giant raspberry. The numbers indicate depth of root in cm. The dotted line indicates the above-ground spread of the canes. N = next plant in the row.

Several plants were found that were not connected with the mother plant ; this may be explained by the brittleness of the roots causing them to snap off during cultivation. The digging of " spawn " also tends to sever roots. This isolation might prove an advantage in cases of later virus infection, as it would lessen the likelihood of underground spread of the virus from plant to plant.

Position of young rootlets.

It was quite easy to see the difference between new growing rootlets and old ones ; the former were succulent and of a white colour, the latter brownish and thinner as can be seen in Fig. 12, Plate IV. These observations are in accord with those

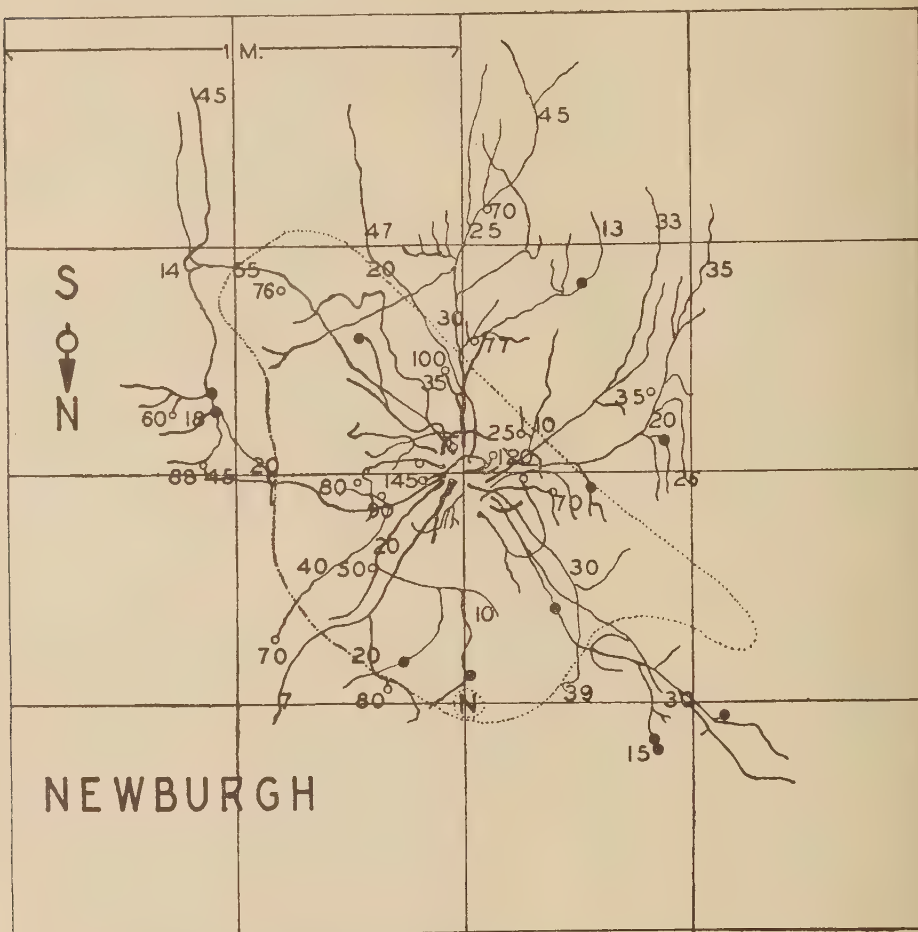

Fig. 2.

Root plan of Newburgh raspberry.
Key as in Fig. 1.

of Rogers (1934), for apples, who found that the new roots were succulent and thicker at first, but later on sloughed off their cortex and became lignified. The small rootlets were found rather intermittently along the roots of the raspberry, tending to be more numerous in certain areas, while in others none was found.

Fig. 3.

Root plan of St. Walfried raspberry.
Key as in Fig. 1.

The largest quantity of rootlets was always found near the crown of the plant, especially in old plants; and many roots showed a big concentration of rootlets at their tips. The rootlets usually grew from all points around the root, but their subsequent disposition in the soil tended to be more horizontal than vertical.

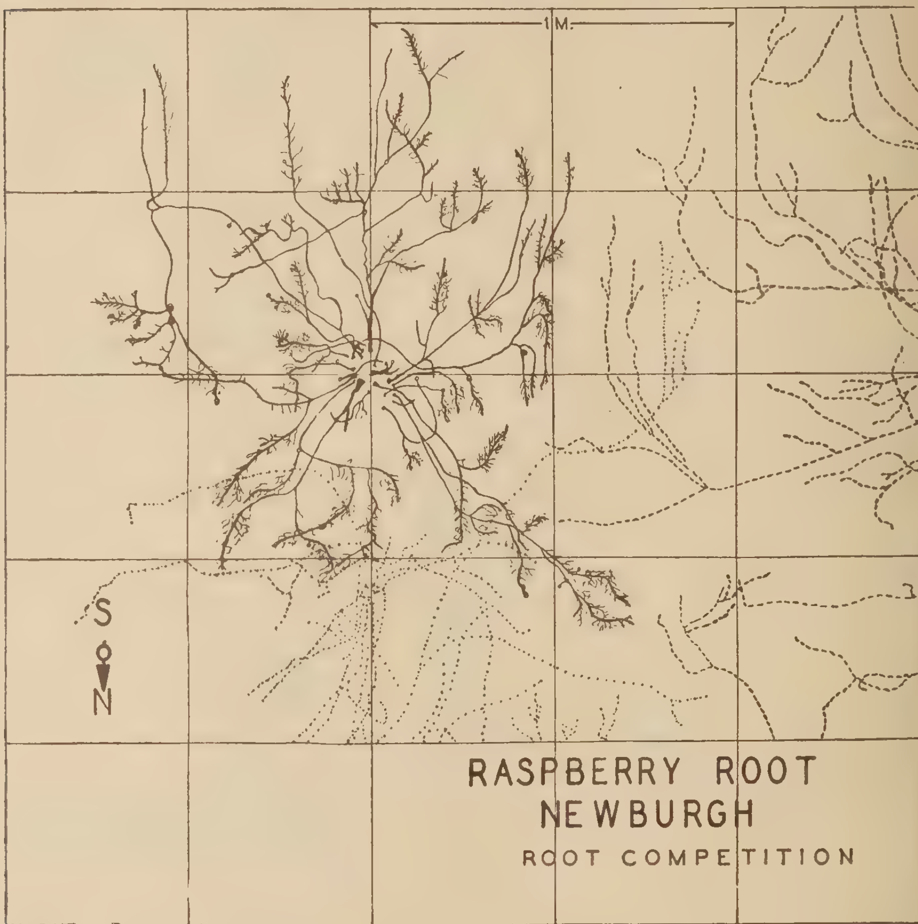

FIG. 4.

Root plan of Newburgh raspberry (solid lines) and neighbouring plants (dotted and broken lines), showing root intermingling and competition.

ADVENTITIOUS SHOOTS.

Some of the raspberry roots seemed to have a tendency to produce suckers, while others lacked that quality. The suckers were usually found on portions of roots growing vertically or at a sloping angle rather than on horizontally growing parts. They were especially common in places near a point at which a root had suddenly changed its direction, and in areas where rootlet production was prolific.

The sucker shoots were found at different depths, varying from a few millimetres to as much as 90 cm. (Fig. 13, Plate V). Although in many instances there was only one shoot on one part of a root, it was common to find two, three, or more and this would point to the possibility that the cause of the production of "spawn" is rather localized.

The roots that gave rise to adventitious shoots were not necessarily large ones, in fact it was possible to find rather small roots supporting big, stout shoots, which, initially, were quite watery. The shoots start their own root system at an early stage of development. The first root rudiment was observed when the shoot was less than 5 cm. long and was not located on the shoot itself, but below the point of union of the root with the shoot ; in later stages roots arose from the shoot itself.

The new root system begins to grow speedily when the first leaves are formed, developing especially in length and spread. In young plants the roots are proportionally longer than in older plants, but the latter have a larger quantity of fine rootlets, especially near the crown.

VARIETAL DIFFERENCES.

Newburgh had relatively less root development than the other two varieties, and the roots were more fibrous and superficial than those of St. Walfried. The adventitious shoots in Newburgh were shorter than in the other varieties and also less numerous. This would be a disadvantage if it were not for the shallowness of the shoot-bearing roots. In the excavated plants of this variety there was no sign of root competition, even though the roots of different plants were intermixed freely in the soil (Fig. 4).

St. Walfried produced the highest concentration of successful suckers, which resulted in a practically continuous line of plants along the row and a very definite intermixing of the roots of different plants.

Norfolk Giant produced, without doubt, the largest plants, and these had the greatest root spread. The roots of this variety showed a marked tendency to develop adventitious shoots near their tips. This character is of definite importance when planning variety trials, since it might lead to a mixture of varieties. Though having the greatest spread of roots, Norfolk Giant is rather a shallow rooted variety, as can be seen in Fig. 7, Plate II, and from Table I.

SUMMARY.

Detailed excavations of the root systems of mature plants of the raspberry varieties Norfolk Giant, Newburgh and St. Walfried are described and illustrated.

The roots grew at varying depths, ranging from 5 to 175 cm. About 70 per cent. of the total root weight was in the upper 25 cm. of soil.

The maximum root length recorded was 180 cm. Suckers may arise from great depths. Attention is called to the importance of wide separation of plants in variety trials.

ACKNOWLEDGMENTS.

The author wishes to express his gratitude to Dr. W. S. Rogers for his advice, constant help and constructive criticism. The plants were kindly provided by Mr. N. H. Grubb and to him thanks are also due for many helpful suggestions.

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PLATE I.

FIG. 5.
Newburgh raspberry in course of excavation, showing general layout of excavation trench.

FIG. 6.
Closer view of Newburgh raspberry in course of excavation, showing roots at varying levels, and (in foreground) sucker shoots arising from a depth of 30 cm.

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FIG. 7.
Reconstructed excavated root system of Norfolk Giant raspberry. Note relatively shallow root system, with some vertically descending roots. Suckers, arising near the tips of shallow roots, are prominent. The length of the measuring rod is 1 metre. (Photograph taken from slightly above original ground level.)

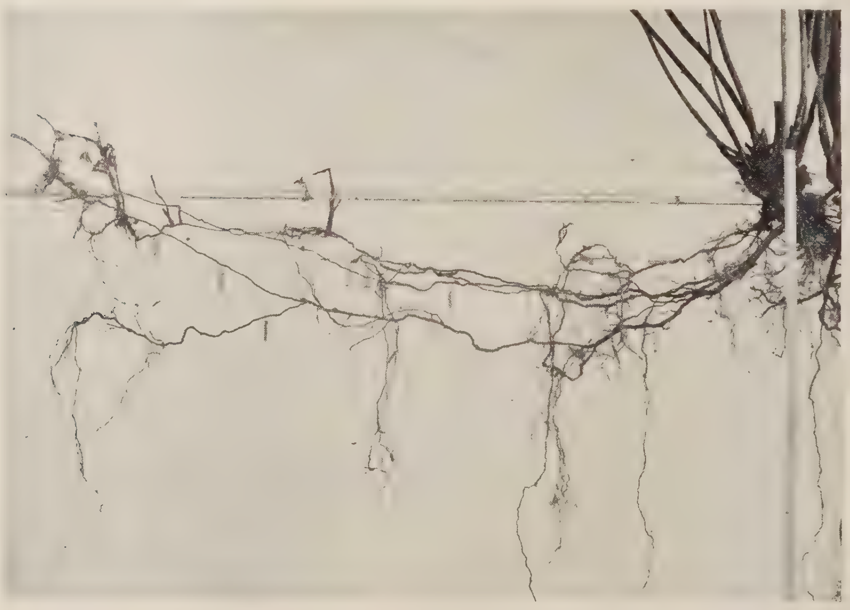

FIG. 8.
Closer view of part of Norfolk Giant raspberry root system. Note the undulation of the roots through various depth levels, and the vertical "sinker" roots.

PLATE III.

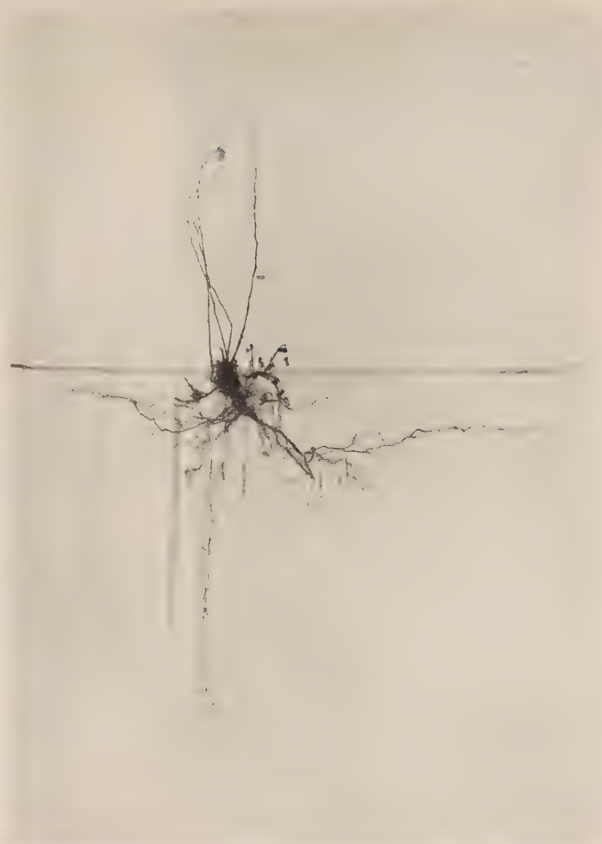

FIG. 9.

Reconstructed excavated root system of Newburgh raspberry. Note roots descend rather more deeply than those of Norfolk Giant.

FIG. 10.

Reconstructed excavated root system of St. Walfried raspberry.

PLATE IV.

FIG. 11.

Roots of Newburgh raspberry growing through an old decayed plum root.

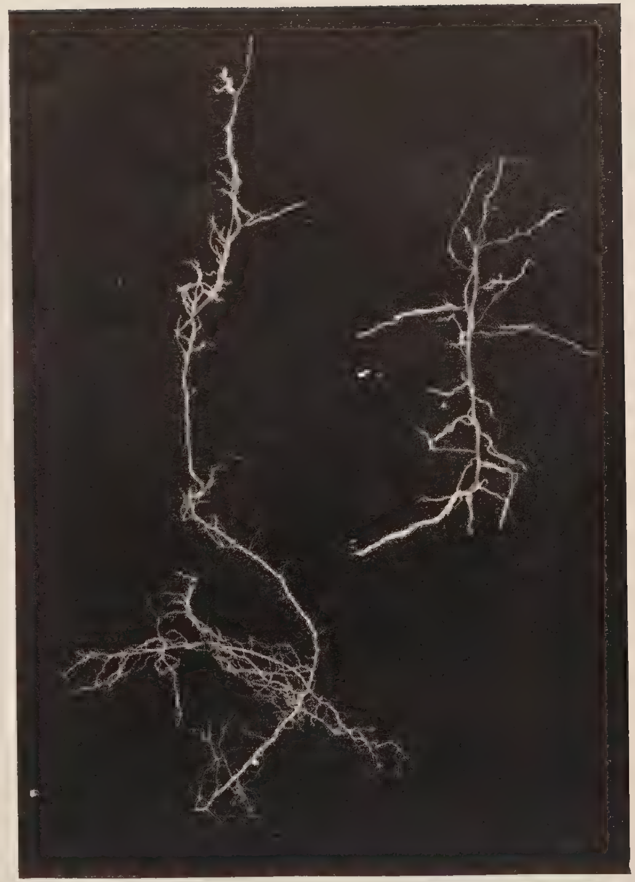

FIG. 12.

Roots of St. Walfried raspberry. Some succulent white, young rootlets, larger than the slightly older roots, are visible.

PLATE V.

FIG. 13.
Roots of St. Walfried raspberry, with sucker shoots arising from a depth of 90 cm. The tips of the suckers were still 50 cm. below ground level when excavated.

INVESTIGATIONS ON THE CONTROL OF THE FRUIT TREE RED SPIDER MITE (*METATETRANYCHUS ULMI* KOCH) DURING THE DORMANT SEASON

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FOREWORD.

The Fruit Tree Red Spider Mite was barely known in fruit plantations here a quarter of a century ago—indeed Theobald (1909) does not mention it as a pest of apples. Since his time this mite has gradually grown in importance, especially in the drier parts of the Eastern and South Eastern Counties where apples are grown commercially. The problem of the efficient control of the pest was one that gravely concerned both fruitgrowers and the manufacturers of the washes they used. Research entomologists and chemists, on the other hand, while appreciating the urgent need for adequate control measures, realized that it might not be possible to satisfy this need until a great deal of fundamental research work had been done ; and this they were anxious to undertake. If it were to be done properly, however, an organization was needed involving collaboration both with growers and manufacturers, and extending beyond the confines of any one particular research station ; for this pest, an infestation of which could not be produced at will, had to be studied in the orchards where it was most prevalent, and attacked in those orchards with resources larger than those usually at the disposal of a research laboratory.

As a result of the common desire for a concerted attack on this pest, a conference was arranged at the Agricultural Research Council's Offices in 1943, under the Chairmanship of Mr. J. C. F. (now Sir John) Fryer, at which were present representatives of the Associated Fruit Growers of Essex (ACE), the Pettar Society of Winter Wash Manufacturers, the Agricultural Research Council and the East Malling Research Station. At that conference it was agreed to initiate a joint investigation into the life history and control of the Fruit Tree Red Spider Mite and the Apple Blossom Weevil. The detailed management of the investigation, which it was agreed to conduct in Essex, was placed in the hands of a Technical Committee, of which Mr. J. D. Edwards of the ACE Growers was subsequently elected Chairman. This Committee included three representatives each of the ACE Growers, the Pettar Society and East Malling Research Station, and one representative of the Essex War Agricultural Executive Committee. This Committee has met as necessary in Essex and reported each year to a conference of all the interested parties in London.

Towards the investigation the A.R.C. has contributed the services and maintenance of the senior research personnel, making a special research grant to the East Malling Research Station for the purpose, and the equipment of a field laboratory in Essex. The ACE growers provided a building suitable for a field laboratory on the farm of Mr. G. V. M. Sketch, and the unrestricted use of the orchards necessary for the conduct of field trials and entomological studies. They have also undertaken all the clerical work in connection with the investigation, and many difficulties have been

surmounted owing to the enthusiastic support of Mr. C. G. Harker, secretary of the ACE Growers, and his successor, Mr. L. Mellor, who also acted as secretaries of the Technical Committee. The Pettar Society have supplied all the spray materials required and have also been responsible for the junior research staff engaged in the work. In the early days one of their members, Plant Protection Ltd., made it possible to make a start by seconding one of their officers, Dr. F. C. H. Gayner, to the investigation until it was possible to appoint a full-time entomologist resident in Essex. Since then the Pettar Society have also provided invaluable extra recording help at peak periods. The E.W.A.E.C. and subsequently the Essex Institute of Agriculture have co-operated by granting facilities at Writtle, including the use of their plantations for field trials. In addition to those represented on the Committee, Dr. D. A. Osmond of the Long Ashton Research Station carried out surveys of the soil on the experimental plantations, while the Meteorological Office has co-operated by lending a sunshine recorder to the investigation.

The history and details of management of this investigation, which is still in progress, have been set out here, since they indicate a new advance in collaborative research, while this is the first major report on its results. This foreword is not the place to mention all those who are contributing to its success, but an acknowledgment of their help will be found at the end of this paper.

Introduction.

The Fruit Tree Red Spider Mite (*Metatetranychus ulmi* Koch)* is now regarded as a major pest. An orchard badly infested by it often produces inferior, muddy coloured and undeveloped fruits and premature defoliation; the latter causing weak fruit bud development. This state of affairs becomes very apparent when an attack persists season after season. The direct injury caused by the mite is difficult to assess in the field because it may be influenced by the state of vigour of the tree, the climatic conditions prevailing and the nature of the soil. It may even be confused with the effects of any or all of these three. The last named applies particularly to Essex where much fruit is grown on the " hot ", dry, gravelly soils.

Many field spraying trials have been undertaken in an attempt to control the mite, but none of them has proved entirely satisfactory for several reasons. A comprehensive study of the life-cycle of the mite had not previously been undertaken in this country, and this work was essential before detailed field spraying trials could be planned. Again, a reliable method of assessing the results of trials had yet to be devised, since it was recognized that the old empirical one of comparing sprayed and unsprayed trees in the field during the summer months proved quite inadequate. It is now recognized that at least two important factors must be observed when assessing field trials ; they are : first, the migration phase of the mite and second, the effect of predators upon mite populations.

To facilitate this work a field laboratory situated at Porter's Farm, Great Braxted, Essex, and used as headquarters throughout this investigation, has proved of inestimable value, largely because it is placed in one of the main fruitgrowing districts of Essex. It consists of a small room located inside the farm fruit packing shed, and is equipped with a bench, a sink and a minimum of apparatus required

* Also known by the scientific names *Oligonychus ulmi* C. L. Koch and *Paratetranychus pilosus* C. & F., but the name used in the text is considered the correct one.

for the work. Storage facilities for spraying screens, experimental washes, etc., are also available at Porter's Farm.

ECONOMIC IMPORTANCE OF THE FRUIT TREE RED SPIDER MITE.

Brief reference to the literature shows that the Fruit Tree Red Spider Mite may cause considerable harm to fruits, particularly apple, and to plum and damson ; but it is also generally accepted that the loss of crop caused as a direct result of mite attack is difficult to determine in the field. Geijskes (1938) considers that the harm due to the red spider mite is difficult to estimate, but states that it is certainly very considerable. Caesar and Ross (1921) provide figures showing differences of crop between sprayed and unsprayed trees, and they also record that the fruit on infested trees is dwarfed. Undersized fruits on infested trees are also noted by Listo (1939), by Frost (1919) and by Garman (1923) in Canada. Loss of fruit size and weight are also referred to by Ross and Robinson (1922).

Bourne (1926) considers that an early fruit drop may also be ascribed to mite attack, and Hammer (1943) records leaf and fruit drop as an indirect result of such attacks. Ross and Robinson (1922) refer also to the check to wood-growth, while Cory (1923) records a reduction in the size of fruit buds. De Ong (1919), discussing the effect on fruit buds, suggests that there is some reduction of sugar and starch in attacked leaves which later renders the trees more liable to frost injury. Newcomer and Yothers (1927) note a subtle effect on the fruit buds and on the tree itself, while Cory (1929) records that mite infested apple foliage is more subject to lime-sulphur injury than that of healthy trees. Listo (1939) mentions a delay in growth formation due to a previous mite infestation.

Kearns and Martin (1940) record that the vigour of the trees is impaired and refer to the cumulative effect of mite infestations, whilst Hey (1944) observes that infested trees may bear late maturing green fruits. Massee (1927) draws attention to the check in growth sustained by plum trees.

The characteristic appearance of attacked leaves is repeatedly referred to in the world literature, one of the earliest records being made by Theobald (1911). Geijskes (1938) in Holland, and Trägårdh (1915) in Sweden, figure cross sections of leaves to illustrate the cell injury caused by the mite, and McGregor (1913) figures comparable cross sections with reference to damage to cotton plants by an allied species *Tetranychus bimaculatus* Harvey.

It will be seen, therefore, that a severe attack of the Fruit Tree Red Spider Mite may play an important part in the economy of the fruit tree in Europe, America and Australia. In Britain it is far more important in Essex and the South-Eastern counties than elsewhere, while it is increasing each year in the Cambridgeshire district. It is not so persistent in the fruitgrowing areas of the West Midlands, the North of England and in Scotland.

THE FIELD TRIALS.

The three years' programme of field trials drawn up in November 1943 by the Technical Committee was a provisional one designed to compare the merits of a petroleum oil miscible wash and a D.N.C. petroleum stock emulsion wash against the winter eggs of the mite. It was also decided to compare these two winter washes at three different periods of the winter, viz. January, February and March.

The field trials were conducted at the following four centres :—

I. Porter's Farm, Great Braxted.
II. Great Braxted Hall Farm, Great Braxted.
III. Thorn Farm, Danbury.
IV. Essex Institute of Agriculture, Writtle.

Design of experiments.

Several factors, some of which might have been avoided in plantations located and planted up expressly for experimental purposes, influenced the design of these trials.

1. Each trial was carried out on a commercial plantation the size and shape of which, as well as the spacing and method of planting of the trees, had all to be taken into account in the experimental design.

2. In choosing those for trial certain essential qualifications limited the number of suitable plantations. Thus, there had to be (*a*) a sufficiently severe infestation of the mite, (*b*) one variety, Cox's Orange Pippin, common to all plantations in the series, (*c*) a reasonably uniform plant of bush trees, not too closely spaced. Since all these trials were carried out on typical Essex fruitgrowing soils, uniformity in this respect was impossible, and the plots had therefore to be large enough, and the treatments well enough replicated, to counteract the effect of patchy variations in soil. (See Appendix I.) To the conditions considered essential at the start might well have been added freedom from excessive risk of damage by spring frosts, since such occurrences have, during the period under review, seriously limited the information that could be obtained on the effects of mite damage on the size of crop and quality of the fruit.

3. As pointed out elsewhere, with high pressure spraying some spray drift is inevitable, however calm the day chosen for spraying. Screens can prevent the drift reaching neighbouring plots to some extent, but not entirely ; moreover, as in other trials for pest control, there are cogent reasons why each experimental tree should be surrounded by other trees receiving identical treatment. Plots had therefore to be large enough to allow of adequate guards.

Number of replications. In each of these trials there were six treatments, plus controls receiving no petroleum spray. Five replications of each treatment were aimed at, but in some instances only four were possible.

Size of plot and number of trees recorded. In all trials each experimental unit consisted of 24 trees arranged 6×4, or 25 trees 5×5. This allowed for a plot of eight or nine experimental trees separated from its neighbour by a double guard row. All the trees in the 24 or 25 tree unit were sprayed alike, but records were confined to the experimental trees in the centre.

X	X	X	X	X	X	X	X
X	C	W	X	X	W	C	X
X	C	W	X	X	W	C	X
X	C	W	X	X	W	C	X
X	C	W	X	X	W	C	X
X	X	X	X	X	X	X	X

Centre I

or

X	X	X	X	X	X	X	X	X	X
X	P	O	O	P	X	O	P	O	X
X	O	O	O	X	X	O	O	O	X
X	O	O	O	X	X	O	O	O	X
X	P	X	X	P	X	X	P	X	X

Centre IV

In most of these plantations, the trees were arranged so that there was one pollinator variety in the centre of eight Cox's Orange. Thus in the 5×5 tree plots, one tree in every nine experimental trees was of the pollinator variety; but at Centre I there were alternate rows of Worcester Pearmain and Cox's Orange, so that each plot contained four trees of each variety. Only at Centre I were records made of both varieties, observations being confined to Cox at the other three.

In the first season eight sample leaves were taken from each of the available eight trees, but after it had been shown that there was no excessive tree to tree variation in the incidence of the mite, the size of the sample was doubled and the number of recorded trees reduced to four. In selecting these trees, undersized or damaged trees were first discarded and the four trees for record drawn at random from the remainder. At Centre I, where there were four trees each of two varieties, sixteen leaves were taken from all the eight trees in the plot in 1945 and 1946, thus giving sixty-four leaves of each variety.

Arrangement of plots. The six treatments were arranged in either four or five randomized blocks in each trial. The control (tar oil only) plots were arranged separately either as a strip of plots running right through the centre of the experiment or in two blocks one at each end. In spraying trials, where all treatments may be expected to give some control of the pest, it often happens that in the result the unsprayed trees give numbers of insects present or percentage hatch very different from those of the sprayed ones. If this is so, while it is important to get as precise a comparison between the treated plots as possible, the comparison of all with the unsprayed trees need not be quite so accurate. Further, by separation of the widely different controls, "transformation" of the data (see Appendix II) may sometimes be avoided. A comparatively large block of unsprayed plots also provides a useful location for studies of the life history of the pest carried out parallel with the spraying trial.

The complete plan of one of the trials (Centre IV) is given in Fig. 1. Points of interest that arose in the statistical analyses of these trials are commented upon in Appendix II.

Treatment of trees.

All the control trees and those scheduled to receive the petroleum oil miscible wash were sprayed with tar distillate miscible oil wash at 6 per cent. to control aphides, sucker and scale insects. This was admissible since it is well-known that the latter wash is not toxic to the eggs of the mite (Massee, 1928). The plots selected to receive the D.N.C. petroleum stock emulsion wash were not sprayed with a tar distillate wash.

In the first season (1943-44) the March application of D.N.C. was made two weeks earlier than the petroleum; but in the two following years both sprays were applied on the same day at each centre.

The trials at Writtle (IV) and Danbury (III) were not continued after the first season because the egg population was too low. The predaceous capsid bug, *Blepharidopterus angulatus* Fall. was largely responsible for reducing the mite population at Writtle. The trial at Great Braxted Hall was discontinued after the second year, also because of the paucity of winter eggs, again due largely to the work of predaceous insects. At Porter's Farm the trial was carried out for three years as originally planned.

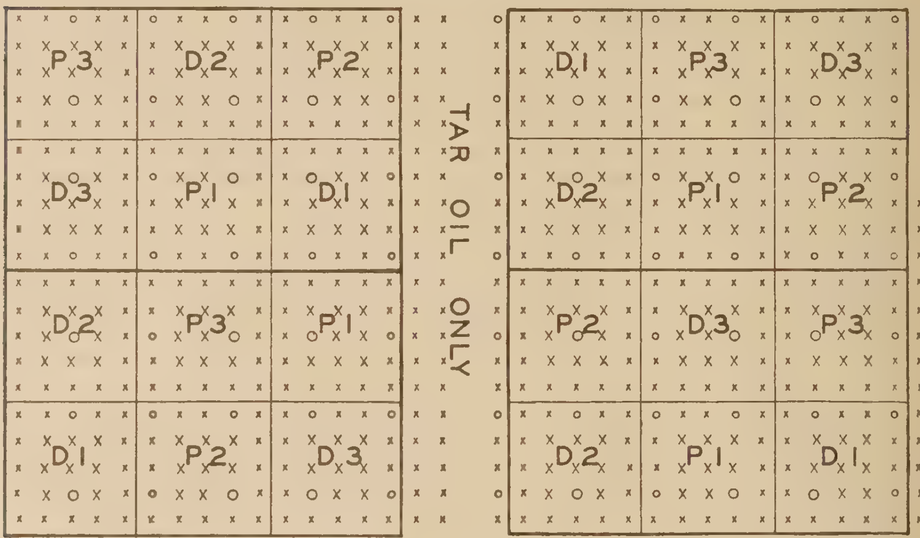

FIG. 1.

SPRAYING EQUIPMENT.

At Centres I, II and III the spraying equipment consisted of a central stationary spraying plant, with permanent underground mains, as used for the routine application of sprays on these farms. The pressure developed at source was about 400-450 lb. per square inch, giving a pressure of approximately 350 lb. per square inch at the nozzle. The spray lances were the Turnbull type fitted with double nozzles (No. 2) and six-hole swirl plates. The lengths of hose-pipe, connected with stand-pipes in the orchards, were sufficient to allow manœuvrability within the randomized blocks of trees. Two or three plots were sprayed at one time by experienced operators, thus ensuring that spraying was completed without undue delay.

A mobile power sprayer, which developed a pressure of 400 lb. per square inch, and fed two nozzles similar to those used in the other trials, was used at Centre IV. The tank capacity of this machine was sufficient to enable all the plots of any one treatment to be sprayed, thus avoiding delays for refilling. At each Centre the petroleum spray was invariably applied before the D.N.C.

The spraying was undertaken by experienced and efficient operators, and each application was made under supervision to ensure that the trees were thoroughly well wetted.

Approximately 300-350 gallons of wash were applied per acre, the amount generally used in commercial practice in Essex.

ROUTINE SPRAY PROGRAMME ON TRIAL PLOTS.

Wettable sulphur was applied as and when necessary after the blossoming period, to control Apple Scab, on all the experimental plots. In addition, nicotine and lead arsenate were used when needed to control Apple Sawfly and Codling Moth. In March, 1946, DDT was applied to control Apple Blossom Weevil. Lime-sulphur

TABLE I.

Showing dates of application of washes, 1943-46.

	Centre.	Tar oil.	Petroleum.			D.N.C.		
		Dec.	Jan.	Feb.	March.	Jan.	Feb.	March.
Winter 1943-44	I	21st	19th	15th	24th	19th	15th	7th
	II	21st	12th	14th	23rd	12th	14th	7th
	III	20th	12th	14th	23rd	12th	14th	6th
	IV	20th	12th	9th	23rd	12th	9th	3rd

	Centre.	Tar oil.	Petroleum and D.N.C.		
		Dec.	Jan.	Feb.	March.
Winter 1944-45	I	12th and 14th	15th	14th	12th
	II	14th	15th	14th	12th
Winter 1945-46	I	11th	15th	12th	11th

applications were not used on any of the experimental plots because of its toxicity to the adult and immature mites.

SPRAY MATERIALS.

1943-44. Owing to the late start of the work in the first season it was not possible to have spray materials specially prepared and those used were drawn from manufacturers' stocks. They were:

Petroleum Oil Miscible Wash. A commercial product of the usual type, containing the petroleum oil specified below together with cresylic acid and mixed resin and fatty acid soaps.

D.N.C. Petroleum Stock Emulsion Wash. A commercial product of the casein-emulsified type with a low content of ammonia. The petroleum oil, in which 2·14 per cent. weight in volume of dinitro-o-cresol was dissolved, was identical with that used in the petroleum oil miscible wash.

Characteristics of petroleum oil.

Specific gravity: 0·907 at 15·5/15·5° C.
Viscosity: 196 secs. Redwood 1 at 70° F.
Unsulphonated residue: 73 per cent.
Distillation: 10 per cent. distilled at 340° C.
50 ,, ,, ,, ,, 368·5° C.
80 ,, ,, ,, ,, 394° C.

These washes were applied at concentrations calculated to give 5 per cent. oil by volume in the diluted wash. The corresponding amount of dinitro-o-cresol in the diluted D.N.C. petroleum wash was 0·107 per cent.

The D.N.C. petroleum wash was far from satisfactory in use. When the drums were opened it was found that large "mushroom growths" had developed on the inside, especially in the angles between the sides and ends and along the internal grooves. These accretions could not be dispersed under field conditions, and large

quantities accumulated on the filters. Some of the larger masses were dark where they had been in contact with the drum and traces of free oil had separated. Evidently reaction had occurred between the liquid and the metal, resulting in deterioration of the emulsion. Analysis showed, however, that the composition of the still free-flowing portion of the wash was not appreciably altered.

On the tree, the wetting and covering power of the D.N.C. petroleum wash was not good and compared unfavourably with that of the petroleum miscible oil wash.

1944-45 and 1945-46. Because of the circumstances of their preparation it was impossible to repeat exactly in 1944-45 the composition of the washes used in 1943-44. Oil from the same original stock was not available and under the prevailing conditions it could not be matched. To safeguard the future position arrangements were made to lay in a stock of oil that would be sufficient for at least three years of field trials on the scale contemplated. It was agreed that the risk of changes in the oil during prolonged storage would be negligible in comparison with the near certainty of considerable variations in fresh supplies from year to year under war conditions.

Since changes in the washes were thus inevitable, the opportunity was taken to modify the formulations with a view to improving the D.N.C. petroleum wash, and to making the concentrations of petroleum oil the same in all the washes at a level that would permit easy measurement of the required quantities in the field.

Petroleum Oil Stock Emulsion Wash. The same petroleum oil with a mixed ammonia casein-potash soap emulsifier.

D.N.C. Petroleum Stock Emulsion Wash. A solution of 2·14 per cent. weight in volume of dinitro-o-cresol in the same petroleum oil, emulsified as above stock emulsion wash.

Characteristics of petroleum oil.

Specific gravity: 0·92 at 15·5/15·5° C.
Viscosity: 200 secs. Redwood 1 at 70° F.
Unsulphonated residue: 75 per cent.
Distillation: 10 per cent. distilled at 353° C.
50 ,, ,, ,, ,, 375·5° C.
80 ,, ,, ,, ,, 395° C.

All three washes were diluted for use to give again 5 per cent. oil by volume and the diluted D.N.C. wash contained 0·107 per cent. dinitro-o-cresol.

Trouble was met with in the miscible petroleum wash in 1944-45. The concentrate, in spite of having a lower oil content than the wash used in the previous year, was very viscous, coming from the drum in large clots, and could be dispersed only with difficulty. Nevertheless, it gave in the end a satisfactory emulsion. A small amount of dinitro-o-cresol salt separated from the D.N.C. concentrate, doubtless because of the high alkalinity, but was easily re-incorporated. This wash flowed freely and when diluted gave excellent wetting and cover on the trees. No difficulty was encountered in subsequent seasons.

The subsidiary spray materials used on the trial plots were:

Tar Distillate Miscible Oil Wash. A standard commercial product meeting the official specification, used at 6 per cent.

Colloidal Sulphur. A commercial product containing 51·6 per cent. sulphur and used throughout the season at 0·5 per cent. in conjunction with 0·2 per cent. of a lime-casein type spreader.

SCREENS TO PREVENT SPRAY DRIFT.

One of the chief practical problems in a spraying trial of this nature is the prevention of spray drift from one plot to another. This problem becomes more acute when high pressures are used and when windy conditions prevail.

Several kinds of screens have been tried out; for example, materials consisting of plain unproofed hessian, waxed and rot-proof green jute, barrage balloon fabric and water-proof hessian have been used at various times in a search for a reliable screen. Of these materials the water-proof hessian proved most satisfactory, being light in weight, non-absorbent and less inclined to "billow" in a moderate wind. (Fig. 2, Plate I).

The design of the water-proof hessian screen was as follows. Length 30 ft. by 8 ft. in depth, and supported by three poles each 11 ft. high, one pole being fitted into a sleeve at each outside edge and the third in the centre. Experience shows that it is essential to use good, stout oak poles for this purpose. A guy-rope is attached to the top of each pole to help to steady the screen against wind pressure.

In normal spraying conditions a team of three to four persons is required to manipulate each screen if spray drift is to be completely avoided.

MITE POPULATION RECORDING.

The search by entomologists in various parts of the world for a reliable method of recording mite populations in the field is conditioned by the fluctuating numbers of mites, which at times may be extremely high on individual leaves; and the fact that mites tend to walk off the leaves once they are picked adds to the difficulty. This phenomenon has been noted by Venables and Dennys (1941). Speed of sampling and rapid examination of leaves are thus urgent considerations when the mites are active. Various methods have been tried by a number of workers. For instance, Jones and Prendergast (1937) evolved a method of obtaining an index of field populations of the Citrus Mite (*Paratetranychus citri* Mc.G.) which entailed the picking of the mite-infested sample leaves and preserving them in 5 per cent. formalin. Later, Henderson and McBurnie (1943) described an electrically operated apparatus for brushing the mites and eggs of *P. citri* Mc.G. from leaves and fruits on to a glass disc coated with newly-applied varnish; the counts being made subsequently. Baten and Hutson (1943) estimated the populations of *P. citri* Mc.G. on portions of sample leaves, the operation being made while the mites were still active. These and other more original methods have been tried out and all proved unsuitable to the requirements of the Essex experiments.

IMPRINT RECORDINGS.

At the start of the investigation it was recognized that any records of mite populations would have to be examined in the Field Laboratory, since it was evident that a relatively large number of leaves would have to be analysed. Further, as population fluctuations within relatively short periods were likely, it was equally important that sample leaves should be collected as quickly as possible to eliminate this source of error. For this reason the examination of individual leaves by hand was impracticable.

A method described by Venables and Dennys (1941) was therefore tried. These workers obtained data of orchard populations of *P. pilosus* C. & F. and also a related species, by placing leaves between two sheets of white mimeograph paper, and then

passing the sheets between the rollers of a clothes wringer. The pressure of the rollers crushed the mites and the eggs on the leaves leaving characteristic and identifiable stains on the paper.

A considerable modification of this method has been worked out, and has been used throughout these trials. By this method of recording it has been possible to obtain a complete collection of sample leaves from all the plots at one centre on the same day. (Fig. 3, Plate II.)

In the first year a standard household wringer with rubber rollers was used; later, second-hand wringers were reconditioned and adapted for the purpose. The apparatus was standardized as far as possible. The "hardness" of the rubber rollers was made to the specification of British standard 40, and the spring-loading of the rollers was capable of field adjustment. The wringer was partly sunk into a small table to allow the folded paper to be fed into the rollers at table level.

A cloth screen was placed around three sides of the table to help to reduce wind interference. A piece of thin cardboard was placed beneath the sheets of folded paper before passing them through the rollers to keep the leaves rigid and prevent their puckering. The apparatus was set up inside the field plots for speedy sampling. Two types of paper have been used for pressing the leaves. The first was that used for "manifolding" work in offices. This paper was eventually discarded because of its poor quality, uneven colour and number of flecks on the surface. The second kind of paper,* now used exclusively, is a high grade one with a glossy and absorbent surface, and the colour is uniform.

The size of the "imprint" paper is approximately 8 in. × 13 in. and when folded longitudinally down the middle it holds eight basal rosette leaves. These are arranged with the lower surface upwards on the right hand side of the paper. The leaves are discarded after passing between the rollers. The "squashes" or "imprints" of the mite eggs are relatively small and pinkish in appearance and are readily distinguished from those of the immature and adult mites. Squashes of thrips, aphides and other small insects sometimes occur, but are quite distinct from those made by the mites and their eggs. This method of recording mite populations has several points to commend it. Many records can be made on the same day and since the imprints are semi-permanent they can be examined at leisure.

Winter egg density.

A tree to tree record of the winter egg density was made at each Centre. This record was obtained at the end of October and in November at the completion of egg laying. It was noted that winter washes tend to darken the mite eggs and make them less conspicuous, so this record was made prior to their application.

Four categories were used for assessing the egg population density. They were as follows :—

0. *No eggs :* No eggs visible on shoots or spurs during examination.
1. *Light Infestation :* Eggs on spurs and a few around buds of one-year-old shoots.
2. *Medium Infestation :* Eggs on spurs and internodes of one year old shoots.
3. *Heavy Infestation :* Numerous egg clusters on undersides of shoots and branches, giving affected parts of trees a bright red appearance.

* "Devon Valley Parchment" Cream Wove—in part rag. Double foolscap 20 lb. per ream of 500 sheets.

The examination of the trees was made in bright, sunny periods only, when the eggs show up more readily. Fig. 4 illustrates a typical record.

SAMPLING RECORDS.

It was essential to obtain a record of the mite population from the trees sprayed the previous winter before the first summer generation of mites had hatched, because the populations of the latter relate to the build-up of the mites during the summer months and not to the percentage hatch of mites after the application of the winter

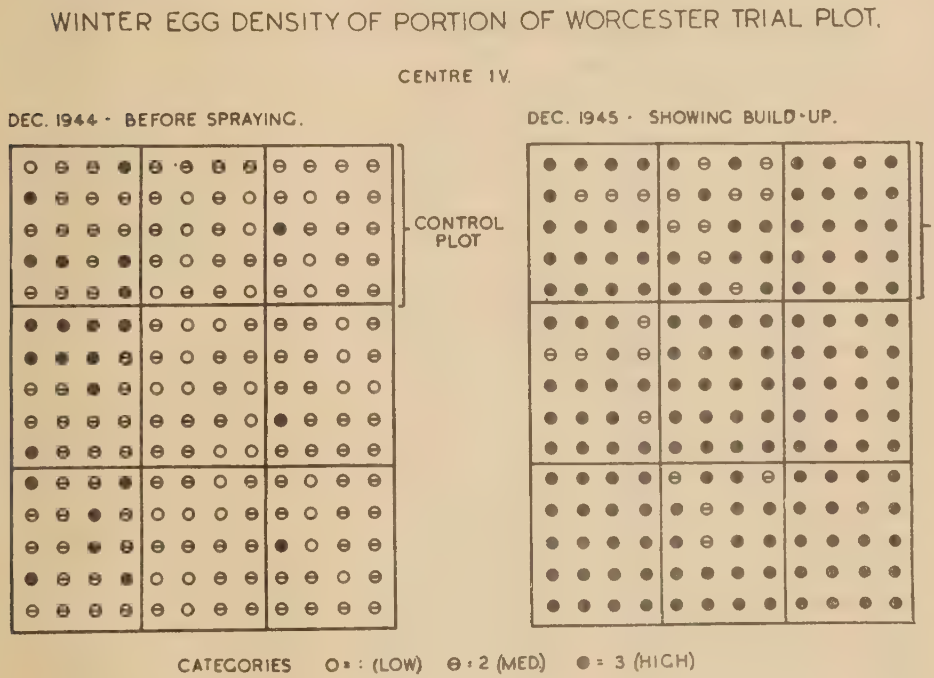

FIG. 4.

sprays. A reliable sampling method has been worked out for this purpose and it has been used throughout these trials.

To standardize the sampling, only basal rosette leaves were collected and they were picked at a height of from 3 ft. to 5 ft. above ground. The same number of leaves were picked from the inner and outer branches of each tree. The leaves were obtained at random from the north, east, south and west aspects of each tree and, without examination, were put into a glass-bottomed " pill-box " and labelled. To ensure that the leaves did not curl or dry up and to minimize the migration of the mites from the leaves to the sides of the box, the " imprint " records were made directly the sampling of a plot had been completed. The time occupied in sampling a plot (sixty-four leaves) was about ten minutes, giving ample time for the operator at the wringer to press the leaves before the next batch was ready. A light shower

of rain during sampling causes little inconvenience, but the leaves should be reasonably dry when collected otherwise the "imprint" records may be of doubtful value. The special paper used for this operation absorbs a little moisture, which has a tendency to spread over the surface.

Determination of completion of hatch of winter eggs.

To determine the date when the first leaf sampling should be made, daily observations were made on the plots from the middle of April to obtain data on the

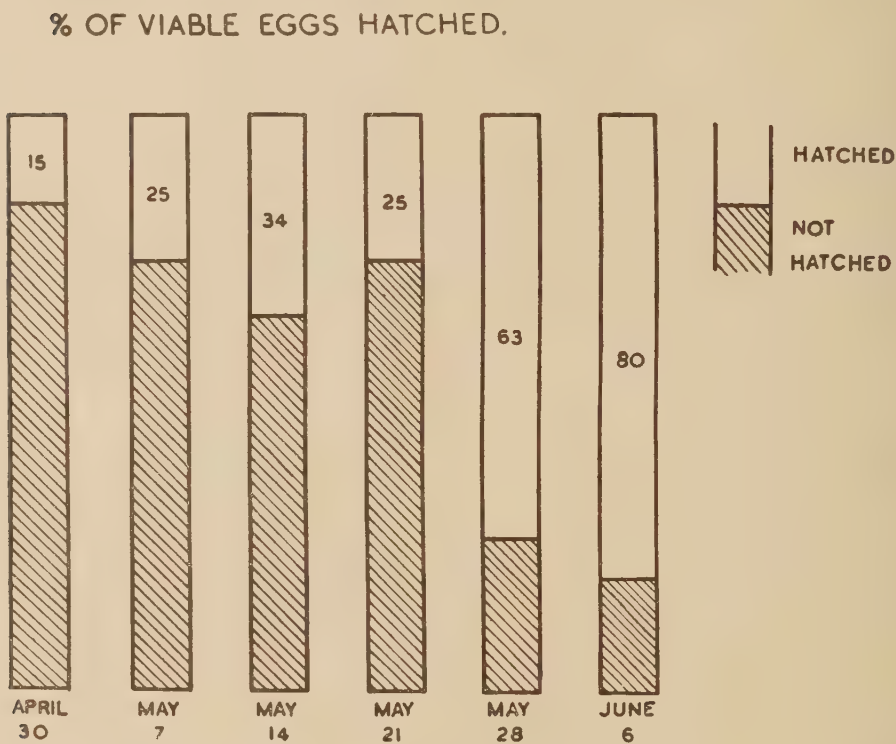

Fig. 5.

hatching of winter eggs. Directly hatching was observed a random sample of twenty-five shoots of the previous autumn's growth were collected from the control trees each week until hatching was completed. The leaves were removed from the shoots which were dipped in water heated to a temperature of 60° C. Each shoot was immersed for one second, the operation being repeated five times. This prevented further hatching of the eggs and enabled the proportion of hatched and unhatched eggs on the date of collection to be determined. The shoots were kept in damp sand and examined at leisure. Fig. 5 illustrates the progress of hatching from April to June.

RESULTS OF SPRAY TREATMENTS. 1944.

In 1944, at each Centre, the March application of petroleum was made approximately two weeks later than that of D.N.C. Table II shows the number of mites per sample leaf following January, February and March applications of petroleum and D.N.C. on Cox and Worcester.

TABLE II.

Numbers of mites per sample leaf following January, February and March applications of petroleum and D.N.C. on Cox and Worcester at four Centres in 1944.

Centre.	When sprayed.	Petroleum.		D.N.C.		Tar oil only.	Result of statistical analysis.
				Cox			
I	Jan.	0·5	0·67	1·0	1·13	12·8	Significant reduction all treatments. No difference between treatments.
	Feb.	0·9		1·6			
	Mar.	0·6		0·8			
II	Jan.	4	3	3	4	8	No significant reduction due to treatment.
	Feb.	2		6			
	Mar.	3		3			
III	Jan.	1·8	1·87	2·2	2·33	3·1	Only March application gave significant reduction.
	Feb.	3·6		4·0			
	Mar.	0·2		0·8			
IV	Jan.	10	7·67	13	10·0	28	Significant reduction all treatments. March application of petroleum gave significantly lower number than Jan. and Feb.
	Feb.	10		8			
	Mar.	3		9			
				WORCESTER			
I	Jan.	4·2	2·53	6·0	3·67	22·8	Significant reduction all treatments. March application gave significantly lower number than Jan. and Feb.
	Feb.	2·6		3·1			
	Mar.	0·8		1·9			

Centre I. It will be noted that the results at Centre I on the variety Cox show a very great reduction of winter egg hatch for the sprays applied at each period; there was a reduction even in January, which was not repeated in later experiments. On Worcester the March application resulted in appreciable control and in significantly less mite infestations than with the earlier treatments. It is interesting also that both the petroleum and the D.N.C. in petroleum sprays gave similar results on Cox, while both were less efficient on Worcester, which had a much higher egg population. There appears to be no obvious explanation for this result.

Centre II. The initial winter egg population at this Centre was low and none of the treatments gave an appreciable control of the mite. This result is indeed difficult to explain since the sprays were similar to those used at Centre I and the spraying operations were performed very efficiently.

Centre III. The winter egg population at this Centre was also very low. The January and February applications effected little or no reduction of the mite

populations. However, the March applications of petroleum and D.N.C. gave a high degree of control and were significantly more effective.

Centre IV. None of the treatments gave complete control, but the March application of the petroleum spray was significantly superior to the other treatments, including the March application of D.N.C.

Comparison of D.N.C. and petroleum in general. On the whole in this year D.N.C. gave slightly less control than petroleum, but this was probably due to the earlier date of application. The problem of spray damage is referred to later.

RESULTS OF SPRAY TREATMENTS. 1945 AND 1946.

In 1945 and 1946 the March applications of petroleum and D.N.C. were made on the same day. Table III shows the number of mites per leaf sample following the January, February and March applications of petroleum and D.N.C. on Cox and Worcester in 1945 and 1946 at Centre I and on Cox at Centre II in 1945.

TABLE III.

Numbers of mites per sample leaf following January, February and March applications of petroleum and D.N.C. on Cox and Worcester at two Centres in 1945 and one Centre in 1946.

Centre.	When sprayed.	Petroleum.		D.N.C.		Tar oil only.	Result of statistical analysis.
				1945 Cox			
I	Jan.	1·7	1·37	2·9	2·0	3·8	Significant reduction all treatments. Petroleum superior to D.N.C.
	Feb.	1·7		1·6			
	Mar.	0·7		1·5			
II	Jan.	1·0	0·5	1·1	0·67	0·7	Only second and third applications superior to control, no difference between washes.
	Feb.	0·3		0·4			
	Mar.	0·2		0·5			
				1945 WORCESTER			
I	Jan.	1·0	0·93	2·9	1·9	3·4	Significant reduction all treatments. Petroleum superior to D.N.C.
	Feb.	1·3		1·1			
	Mar.	0·5		1·7			
				1946 Cox			
I	Jan.	2·7	1·17	1·6	0·8	5·9	Significant reduction all treatments. Later sprayings more effective. D.N.C. slightly superior to petroleum.
	Feb.	0·3		0·3			
	Mar.	0·5		0·5			
				1946 WORCESTER			
I	Jan.	2·0	0·93	0·7	0·43	4·8	As Cox.
	Feb.	0·4		0·3			
	Mar.	0·4		0·3			

The second and third applications only were superior to the control, but there was no difference between washes at Centres I and II on Cox in 1945. On Worcester at Centre I there was a significant reduction with all treatments, and the petroleum was superior to D.N.C. in 1945.

In 1946 there was a significant reduction in number of mites from both treatments; again the later spraying was more successful. However, the D.N.C. applications were slightly superior to petroleum on Cox and Worcester, the reverse of what happened on Worcester in 1945.

1945.

Centre I. In general the February and March sprayings were more effective than the January application, although not always significantly so. The March application was slightly superior to the February spraying. There were fewer mites on the petroleum sprayed trees than on those receiving D.N.C.

The results obtained with the two later applications of both petroleum and D.N.C. were good enough to give a commercial control of the mite.

Centre II. The results at this Centre are of interest since the trees sprayed in January showed no improvement over the controls. The February and March applications of petroleum and D.N.C. did not differ significantly, but both were much superior to the January applications.

1946.

Centre I. The results of January, February and March applications on both Cox and Worcester show a great reduction of mites when compared with the controls. Also the February and March applications were significantly superior to the spray applied in January. The D.N.C. sprays in this year actually gave a greater reduction of winter egg hatch than petroleum.

CONCLUSIONS.

Centre I. The figures for the percentage of viable winter egg hatch show that on the variety Worcester the February and March applications were more efficacious than the January ones in all three seasons. Similar results were obtained on the variety Cox for the 1945 and 1946 seasons: but in 1944, when the mite population in early June on the control trees of this variety was about 50 per cent. lower than on the Worcester controls, the winter egg hatch was greatly reduced by the January spraying. (In 1944 the January sprays were applied a week later at Centre I than at the other Centres.) This divergent result may be due to the initial lower egg population and a consequent better contact of the spray with the individual eggs. In 1945, although the later sprayings were superior to that in January, as in 1944 and 1946, there was generally less reduction in hatch due to spraying than in the other two years. This would not appear to be due to the composition of the sprays as the wash was obtained from the same source for each application.

Also, in 1944, when the experiments were made at all the four Centres, the results at Centre I were appreciably superior to those of the other Centres.

Field observations showed clearly that, despite the reduction in hatch due to spraying (great in 1944, though not so large in 1945), at the end of each season the winter egg density was higher than that of the previous winter. Indeed, the egg density on the sprayed trees in the winter of 1946-47 was higher than in 1943-44. In fact there had been a gradual increase extending over the three seasons. Conversely, after the first two seasons the control trees which received a tar distillate wash only were slightly more infested with eggs than at the beginning of these trials, and this slight increase was much less pronounced than on the sprayed trees. A much greater increase was observed, however, in the winter of 1946-47.

The general tendency is that even when the mites are appreciably checked by the spray treatments, those surviving gradually build up through the successive generations, so that the winter egg density in the subsequent winter may be as high as, or even higher than, that of the previous winter. This build-up of the mite population is important and will be referred to in greater detail later on.

Leaf-bronzing was not very noticeable on the sprayed trees in 1944—and some of the differences due to the treatments were still in evidence during the autumn. In 1945 leaf-bronzing was more severe and, as it occurred considerably earlier in the summer, it evened out the differences between the treatments by the end of July.

This indicates that the build-up occurred much later in 1944 than in 1945. The difference may be very important, for early bronzing of the leaves may seriously weaken bud development.

As a rule, the important predators do not appear on the trees until late in the season, and if the mite population is reasonably low, the predaceous insects will tend to reduce the populations still further.

Centre II. The results at this Centre for two seasons' trials follow the same general trend as at Centre I, the March application being the most efficient. The winter egg density of the trees was relatively low in 1943-44, and there is little evidence that any of the treatments affected the mites appreciably. The winter egg population was somewhat higher the following winter. Predaceous insects, notably *B. angulatus* Fall., were abundant during the two summers concerned, and doubtless were responsible for low mite populations in the autumn. However, two successive winter treatments at this Centre did not permanently reduce the mite populations, although the March application did not considerably delay the build-up. Leaf-bronzing appeared only late in the summer in 1944, but quite early in the season in 1945, in which year the mite populations on sprayed and unsprayed plots rapidly evened up.

Centre III. The egg population of the control trees was very low in 1943-44, which made the results difficult to assess at this Centre. However, it was quite obvious that the results of the March applications were similar to those at other Centres and effected greater control than the earlier spray treatments.

Centre IV. The one year's results at this Centre in 1944 again showed that the March applications were superior to those of February and January. The February sprays, however, did substantially reduce the mite population. Predaceous insects became so numerous at this Centre during the summer and the mite populations were so reduced by their attack, that the trial was not repeated the following year.

General conclusions.

The tabulated results given above confirm the extensive field observations which formed the practical basis of this three-year investigation. In general the following conclusions have been arrived at :—

1. The February and March applications of petroleum and D.N.C. are superior to the January one. There is, however, little significant difference between the mid-February and March applications of both washes.

2. There is no evidence to suggest that the addition of D.N.C. to a petroleum spray increases the efficacy of the wash against the mite ; for, when the petroleum and D.N.C. were applied on the same day, the degree of control obtained was about the same.

3. Even when a substantial reduction in hatch of winter eggs is achieved (say up to 97 per cent.) by petroleum or D.N.C., sufficient viable eggs remain to enable large populations to be built up by the late summer and autumn, which may cancel out any differences previously noted for these treatments, and may result in weaker bud development.

4. Although a very good temporary control may be obtained by winter spraying, the eventual population at the end of the season may be in no way related to it and may be even greater than at the beginning of the season before the spraying began.

THE POPULATION BUILD-UP.

In Essex orchards, where the mite populations have been materially reduced by the application of winter washes, the rapid build-up of numbers during the summer months is striking. Several complete generations of the mite occur annually. In 1945, in Essex, three complete generations and a partial fourth were noted. So many generations invariably lead to a remarkable increase in population ; and these eventually compensate for the initial reduction due to the winter washing.

The winter egg population of the trees in many Essex orchards is so high that the undersides of the main limbs and many of the branches may be covered with red egg masses. This density of egg population must be considered in terms of millions rather than hundreds. Garman (1921) and Newcomer and Yothers (1929) also refer to the very heavy deposition of winter eggs. Therefore, even where a very high degree of control of winter eggs has been achieved—say 95-98 per cent.—many eggs survive. As stated before, although winter control measures may reduce the population in the spring and early summer, a rapid increase in numbers occurs in July, August and September. For instance, Cutright (1939) suggests that even a control as high as 98 per cent. at the beginning of the season is not sufficient to hold *P. pilosus* in check for the remainder of the year. Dean (1942) also refers to the potentialities of a 1-5 per cent. winter egg survival after spraying the previous winter.

This build-up has been very pronounced during this investigation, and the figures given in Table IV, which were obtained in the course of routine recording, supplement the field observations.

The result of this high build-up is sooner or later manifested by the typical leaf-bronzing, which increases in severity until all the foliage may assume a dull

TABLE IV.

Mean numbers of mites and summer eggs on sixteen leaves in June and August, 1944, at two Centres.

Centre.	Variety.	Date 1944.	Mites.	Summer eggs.	Total population.
I	Cox	June	1	0	1
		Aug.	77	133	210
	Worcester	June	5	1	6
		Aug.	92	209	301
II	Cox	June	4	23	27
		Aug.	177	190	367

leaden appearance. Leaf-bronzing has been noted by the end of May, but generally it is not appreciable until July and August. Attempts to assess the success or failure of winter washes by the degree of leaf-bronzing have been made, but cannot be depended upon, since by late summer any broad differences noted earlier may become completely evened out.

A typical example of the mite population build-up occurred in 1945 and 1946 at Centre I and will be evident from the figure given in Table V.

TABLE V.

Mite population build-up at Centre I in 1945 and 1946. Mean eggs and mites per sample of sixteen leaves.

Date of sampling.	Cox.		Worcester.	
	Petroleum sprayed.	Tar oil only.	Petroleum sprayed.	Tar oil only.
1945				
May	27	80	31	76
July	253	266	385	122
1946				
June	12	81	15	108
August	448	541	309	627

The build-up on the trees which received tar oil only during the winter was not so high as on those sprayed with petroleum and D.N.C. in petroleum (except Worcester in 1946). The correctness of this statement is fully supported by the field observations, which show that at times trees supporting an initially high population in early summer may show a marked decrease in late summer and autumn. For example, at Centre IV the field experiment laid down in 1943-44 was abandoned after one year's investigation, because predatory insects, notably the beneficial capsid bug *Blepharidopterus angulatus* Fall. considerably reduced the mite population, as will be seen in Table VI.

TABLE VI.

Mite and egg populations at Centre IV in June and August, 1944. Mean per sample of sixteen leaves.

Date.	Mites.	Eggs.	Total populations.
June	11·5	33	44·5
August	9·5	15	24·5

This reduction, in contrast to increases at other Centres, was followed by an unusually low winter egg population. However, a rapid build-up occurred in the summer of 1945. In some seasons dispersal of mites from neighbouring plantations

may greatly accelerate the build-up, so that despite a good commercial control of eggs from winter spraying the population may eventually be as high as, or even higher than, that of the previous season.

The significance and implications of this build-up are tremendous and cannot be over-stressed.

DIRECT MITE INJURY NOTED IN THE PRESENT TRIALS. LEAF-BRONZING.

The only kind of injury noted in these trials that may be directly ascribed to the mite was leaf-bronzing. The mite feeds on the upper and lower surfaces of the leaves, more especially the latter. Mite infested leaves during early summer exhibit a mottling of a paler hue than the uninfested ones. Later, the infested leaves lose their characteristic deep green, and become dull leaden or bronzed in appearance. The texture of such leaves is " leathery " and leaf-fall often occurs prematurely. A striking example of this occurred in a large Cox orchard near Chelmsford, Essex, where the trees were denuded of their foliage early in August. It is illustrated in Fig. 6, Plate III.

Leaf-bronzing usually starts inside the framework of the tree, where a " housed-in " condition is agreeable to the mites. Occasionally, however, the leaves on the outside branches of a tree may become bronzed early in the season. Complete bronzing of the foliage is common in the late summer and autumn. Severe leaf injury must have a very harmful effect upon the trees, particularly those on the " hot " dry soil of many Essex orchards, where considerable premature leaf-fall occurs every year.

When leaf-bronzing occurred on the same trees each year, as it did on some of the trial plots, a serious cumulative effect was noted, and probably bud development suffered. This damage to the buds could not be measured in these trials, and, indeed, a very careful investigation by a qualified plant physiologist would be necessary to confirm it and estimate its importance.

A record of leaf-bronzing was made at each Centre in September and October. Except in 1944, the general increase of mite populations, exhibited by varying degrees of bronzing, resulted in a complete levelling up of the treatments. However, in 1944 leaf-bronzing records at Centres I and III and to a less extent at Centre II support other conclusions reached in these trials, namely, that the success of the winter wash applications varies according to the time at which they are applied.

At Centre IV in 1944 leaf-bronzing was noted on only a few trees. This was to be expected since the mite population had been greatly reduced by predaceous insects during the summer months.

EFFECT ON CROPPING AND FRUIT QUALITY.

Although the mite is reputed to cause so much harm to the tree, there was no significant difference in the crop weights of the treated and control trees in any of the trials. This fact can be fully explained in the following manner. In each of the seven trials, the mite population of sprayed and control plots evened up by the latter part of July, i.e. at the start of the period when the mite causes most damage to the tree and at a time when the mite still has three complete months in which to feed. Thus, until the mite is completely eradicated from the sprayed trees throughout the whole year, it is unlikely that the effect on cropping will be proved. For similar reasons it was not possible to show any effect of the winter control of the mite on fruit quality.

Spray injury records.

Until the physiological aspects of spray damage on fruit trees have been studied, little progress will be made towards eliminating bud injury in the field. Observations have been made at each trial Centre and the amount of spray damage, if any, recorded by comparing the buds of sprayed and unsprayed trees regularly throughout the late winter and spring. It was then noted that certain types of damage, particularly injury to the buds, became more apparent as the season advanced. Severe frost following a period of relatively warm weather in late winter may mask any symptoms of spray injury, but it may on the other hand increase them ; in either case the primary cause of the injury is difficult to determine. Spray injury was recorded in four categories :

0. Undamaged.
1. Slight retardation of leaves, either rosette or one-year-old wood ; without bud killing.
2. Retardation of buds, a few buds being killed.
3. Severe retardation of buds, many buds killed and obvious leaf scorch.

Table VII shows the spray injury recorded at all the trial Centres during 1944, 1945 and 1946.

1944. The January and February applications of the petroleum and D.N.C. sprays did not cause any significant injury, but at Centre III more serious damage was caused by the application of the March petroleum spray. (See Table VIII.) The reason for this injury was not apparent, but the strong wind prevailing during the spray application, causing the spray drift to cover and dry on those trees already sprayed, may be a direct cause of the damage.

Following the March application the Cox trees at Centre I exhibited more injury than the Worcester, although the latter is usually regarded as the more susceptible variety under Essex conditions.

1945. Neither the January nor the February applications of petroleum and D.N.C. caused injury which could be regarded as serious, and, indeed, on most trees, there was not sufficient to record.

The March applications of both sprays caused more injury than the earlier washes, but this is not important. It should be mentioned that the buds were much more advanced than when the sprays were applied the previous year and exhibited the familiar " scorch " type of damage peculiar to these washes.

Cox was again more susceptible than Worcester at Centre I. (See Table IX.)

1946. For the third year the March applications of petroleum and D.N.C. caused relatively more bud injury than those of January and February, the petroleum wash actually causing more injury than the D.N.C. For the third year the damage was more evident on Cox.

General conclusions on spray injury.

It must be emphasized that the spray injury recorded was not serious or widespread except at Centre III in 1944. On this occasion the March application of petroleum killed many buds and greatly retarded most of the remainder. It may be significant, however, that the petroleum was applied some ten days later (March 23rd) than in the following two years. Neither the petroleum nor the D.N.C. sprays caused any serious damage when applied as late as mid-March in any of these trials, although

TABLE VII.

Spray injury recorded at each Centre.

			1944. Centres I, II, III and IV.	1945. Centres I and II.	1946. Centre I only.
	Date spray applied.	Bud stage at time of spraying.	Main observations made first week of April.	Main observations made late March.	Main observations made late March and late April.
P.	Jan.	Dormant	Negligible retardation on a few trees.	No commercial damage—very slight retardation.	No injury.
	Feb.	Dormant	Marked slight retardation of leaf development both on spurs and on one-year-old wood.	Retardation more severe than with D.N.C. notably at Centre I.	Slight bud retardation.
	March	1944. Breaking 1945. Burst 1946. Delayed dormant	Injury varying from slight retardation to severe with many buds killed (commercially serious at Centre III).	Considerable severe " scorch ", but few buds killed. Retardation noted but not severe.	General slight bud retardation.
D.N.C.	Jan.	Dormant	Negligible retardation on a few trees.	No commercial damage, very slight retardation.	No injury.
	Feb.	Dormant	Marked slight retardation of leaf development both on spurs and one-year-old wood.	No serious damage, some slight retardation of leaf development on spurs and one-year-old wood.	Slight retardation.
	March	1944. Swelling 1945. Burst 1946. Delayed dormant	Retardation on more trees than for the Feb. application and occasionally more marked retardation with a few buds killed.	Considerable severe " scorch ", but few buds killed. Retardation noted but not severe.	Slight retardation.

both these washes are reputed to cause much harm when applied at this period of the year under Essex conditions.

TABLE VIII.

Percentage of trees of Cox in each spray damage category at Centre III in the spring of 1944 under each treatment.

Treatment.	P1	P2	P3	D1	D2	D3
Category 0 ..	71	33	14	100	53	14
Category 1 ..	29	67	41	0	47	81
Category 2 ..	0	0	36	0	0	5
Category 3 ..	0	0	9	0	0	0

TABLE IX.

Percentage trees in each spray damage category at Centre I in the spring, 1945, under each treatment.

	Worcester.						Cox.					
Treatment.	P1	P2	P3	D1	D2	D3	P1	P2	P3	D1	D2	D3
Category 0 ..	100	75	75	100	88	63	88	50	0	88	81	0
Category 1 ..	0	25	25	0	12	37	12	50	63	12	19	0
Category 2 ..	0	0	0	0	0	0	0	0	37	0	0	69
Category 3 ..	0	0	0	0	0	0	0	0	0	0	0	31

MAXIMUM AND MINIMUM TEMPERATURES DURING MARCH AT CENTRE I.

Temperature records were taken during March, 1945 and 1946, at Centre I, covering the period of the third spray application. As the seasons proved to be very different, this application was made at the " bud burst " stage in 1945, and at the " delayed dormant " stage in 1946. Spray injury of the " scorch " type was noted following the petroleum and D.N.C. applications in 1945. No injury was recorded in 1946.

Table X shows the temperature records for the period under review.

It will be noted that the temperature rose to 53° F. in 1945 on the day of the application, and rather high temperatures prevailed for some days afterwards. Because of this, spray injury was recorded on trees of both spray treatments. In 1946 the temperature was ten degrees lower at the time of spraying and no injury was recorded. This may be due to the fact that the buds were still in the " delayed dormant " stage owing to the late season.

TABLE X.

Date.	1945.		1946.	
	Min. ° F.	Max. ° F.	Min. ° F.	Max. ° F.
March 2	28	56	29	36
3	25	48	28	31
4	32	51	25	44
5	30	51	31	35
6	34	48	30	35
7	41	50	31	33
8	39	53	26	38
9	30	55	30	40
10	42	56	27	39
11	40	56	30	45
12	34	48	**38** P. & D.N.C.	**43**
13	**34** P. & D.N.C.	**53**	36 application	49
14	39 application	62	33	36
15	31	68	32	35
16	34	53	33	35
17	41	56	24	40
18	36	64	36	50
19	41	59	44	53
20	40	56	36	58
21	33	59	48	57
22	34	64	44	54
23	38	60	36	49
24	41	58	37	50
25	39	64	32	58
26	44	62	37	57
27	40	44	37	64
28	42	58	37	61
29	—	—	40	53
30	42	64	37	63
31	42	58	35	64
April 1	48	57	34	63
	(Rainfall :	1·03 in.)	(Rainfall :	1·44 in.)

(The readings were taken at 9 a.m. on each day, therefore they refer to the preceding 24 hours.)

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The field records were taken by the staff of the Field Laboratory, Great Braxted and the staff of Members of the Pettar Society.

The photographs reproduced in Plates II and III were taken by Mr. Fred Spalding of Chelmsford.

To all concerned in this investigation, the authors wish to express their very sincere thanks.

SUMMARY.

An account is given of a three-year investigation on the destruction of the winter eggs of the Fruit Tree Red Spider Mite (*Metatetranychus ulmi* Koch) on apple

by means of petroleum and D.N.C. washes applied in January, February and March.

1. Biological data relevant to this investigation are discussed.

2. A mite population recording procedure is described.

3. Measures designed to minimize risk due to spray-drift when treatments are applied to randomized blocks are outlined.

4. It is shown that petroleum and D.N.C. washes applied at the same strength, on the same day, inhibit the hatching of a high proportion of the winter eggs, and that the mid-February and March applications give significantly greater egg-hatch reductions than that of January.

5. The results obtained with petroleum and D.N.C. are similar, no significant differences being noted. The addition of D.N.C. to the petroleum wash did not increase the efficacy of the wash.

6. Even when the hatching of 97 per cent. of the winter eggs has been inhibited by these washes, the mites that hatch from the surviving eggs give rise to enormous populations by the late summer and consequently even out any broad differences previously ascribed to any given treatment.

7. Whilst the number of eggs of the first generation on sprayed trees can be correlated with the measure of winter egg destruction, this correlation cannot be made in late summer when the " build-up " of mite populations has compensated for this initial check.

8. The " build-up " of mite populations occurs annually and is considerable, because there are three or more generations per year in this country.

9. It is considered that a programme of winter and summer sprayings will be necessary to achieve commercial control of this pest.

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APPENDIX I.

NOTES ON SOIL CONDITIONS AT CENTRES I-IV.

The soils of the experimental orchards at Centres I, II and III are derived from materials shown on the Geological Survey Map as sand and gravel, which fringes on the Boulder Clay. In this respect they are typical of the orchard soils of the surrounding areas. The sand and gravel are variable in texture, and in places thick bands of clay occur. The character of the soils is, therefore, complex.

Centre I. The site is practically level and although drainage appears to be satisfactory the profile of the soils indicates that drainage is poor and imperfect.

There are three soil series. One consists of dark brown clay with a fair proportion of quartzite and pebbles and flints, with subsoil of yellow clay with grey and blue-grey mottling containing fewer stones than the surface layer. The second consists of a brownish-grey fine sandy medium loam surface with a fair number of quartzite pebbles and flints which overlie fine sandy medium loam containing about 40 per cent. of stones. The amount of stone and thickness of the layers varies considerably in these two series which collectively cover most of the orchard.

The third series is of very small extent and may be regarded as a variation of series two. The main difference appears to be the coarser texture of the soil through the profile.

Centre II. Situated mainly on a flat area with a gentle slope to the west on the west side with the northern half of the orchard a few feet higher than the southern half. Drainage of the site is generally satisfactory but poor in some parts of the southern half and free or even excessive in some parts of the northern half of the

orchard. Five soil series were identified, with series one and two as at Centre I occupying the greater part of the orchard.

Centre III. This site has a very gentle slope to the north and has satisfactory drainage ; impeded drainage conditions, however, occur in some parts of the orchard. Five soil series were identified.

Centre IV. Sited on flat land with a gentle slope to the south at the south end. Drainage is generally satisfactory with some soil profiles showing signs of imperfect drainage.

The orchard at Centre II includes small areas of undersized trees, so characteristic of Essex soils. These so-called " hot-spots " are of a gravelly nature and are responsible for excessive drainage and drying-out of the soil in a normal summer. The orchards at Centres I, III and IV are uniform and without smaller trees.

Attempts to correlate mite infestations on different soil types form a part of the biological investigation, but broadly speaking it may be said that severe mite infestations are not associated with any particular type of soil within any one centre.

Further, the value of the randomization of the spray treatments is appreciated when one considers the different series of soils of the various trials.

APPENDIX II.

The Statistical Interpretation of Data from Red Spider Mite Trials

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When the above trials were first proposed, the Statistics Section at East Malling had fortunately been in consultation with Mr. G. A. Carter of Technical Products Ltd., about some trials on Red Spider Mite being conducted by that company. Much of the statistical procedure adopted in the present series of trials was based on Mr. Carter's results and this help is gratefully acknowledged. It is the purpose of this Appendix to set out the conclusions on statistical procedure with Red Spider Mite arrived at as the result of further experience.

Size of Sample. In the earlier trials of this series there were eight trees to a plot and eight leaves were taken from each tree, but later sixteen leaves were taken from each of four trees. Since there appears to be little variation in infestation between neighbouring trees, it follows that it is the total number of leaves per plot that determines the sampling error rather than the number of trees sampled ; and the two procedures are, therefore, approximately equivalent. An increase in sample size above 64 leaves per plot would lead to an advantageous diminution of error variation, but it would further increase the already heavy burden of counting egg and mite imprints ; and for that reason it is not recommended as a standard practice.

Means of Reducing Experimental Error. In some trials a visual record of "winter egg density " was made, which explained variation in the subsequent imprint counts that would otherwise have been inexplicable. In this way a useful reduction of error variation was brought about and it is recommended that this record be made whenever possible.

Another attempt to reduce experimental error was made by correcting the infestations of plots by those of their neighbours (Papadakis, 1937 ; Bartlett, 1938). The results were not promising, and the method is unlikely to be of much service

Investigations on the Control of the Fruit Tree Red Spider Mite (*Metatetranychus ulmi* Koch) During the Dormant Season. By M. D. Austin and A. M. Massee.

PLATE I.

Fig. 2.

Screen used to prevent spray drift.

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PLATE II.

FIG. 3.
Wringer used for recording mite populations in the field.

PLATE III.

FIG. 6.

Danbury, Essex. September 3rd, 1944.

Cox's Orange Pippin. Advanced stage of defoliation.

in this type of trial. It may be, however, that any association between the infestations of adjacent plots would have been more marked if the sample had been much larger.

Multiple Analyses of Variance. Since all records were in duplicate, each count of eggs being matched by a count of mites, the experiment was tried of considering the two simultaneously, using the method of Lawley (1938*a*, *b*) ; but no advantage was thereby gained.

Transformation of Insect Counts. Throughout these trials the infestation of a tree has been measured not by the actual numbers of eggs or mites, n, found on the sample leaves, but by $log\ (n+1)$ (Williams, 1937), the intention being to equalize the contributions to the error variation from the various treatments. It now appears that $log\ (2n+1)$, suggested by Pearce (1945), would have been a little better. However, no transformation was found capable of completely equalizing the contributions ; and it is, therefore, recommended that, whenever possible, trials should be designed with sufficient replicates to permit the breaking up of the error sum of squares into components, each appropriate to a comparison in which the experimenter is especially interested. It would be unfortunate if this led to a reduction in the number of leaves forming the sample from each plot ; but even if this were necessary, it would still be worth while to increase the number of replicates.

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THE PRODUCTION OF MOLYBDENUM DEFICIENCY IN PLANTS IN SAND CULTURE WITH SPECIAL REFERENCE TO TOMATO AND BRASSICA CROPS

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THE first proof that molybdenum is an essential element for the nutrition of any of the higher plants was obtained with the tomato by Arnon and Stout (3) in 1939. Piper (15) and Warington (21) showed subsequently that molybdenum is also necessary for cereals and lettuce, and beneficial responses to the addition of molybdenum have also been recorded by Arnon (2), for asparagus, mustard and lettuce, and by Bobko and Sauvina (6), and Jensen and Betty (12), as regards nodule formation in leguminous plants. All these results were obtained in water culture and although the nutrient reagents in some of these experiments were prepared as highly purified solutions (15), (20), the methods used were not specially effective for the elimination of molybdenum, as has been pointed out by Piper (15), (16) and also confirmed in several trials by one of us (E.J.H. unpub.).

This paper describes results obtained for tomato, cauliflower, savoy cabbage and mustard grown in sand cultures, for which the nutrient reagents were purified by a simple method which is effective for the removal of molybdenum ; the method may also be suited for the removal of other trace elements in similar studies.

EXPERIMENTAL.

MATERIALS AND METHODS.

The procedure was as follows: The sand was contained in pyrex glass beakers of five litres capacity, each fitted with a central drainage hole of one inch diameter in the bottom. The hole was covered by glass wool and a small glazed perforated filter disc. The beakers were " steamed " with the vapour of constant boiling hydrochloric acid before use.

The sand was a relatively pure silica sand obtained from Leighton Buzzard, Bedfordshire, 90 per cent. of which was retained between 24 and 40 mesh sieves. This fraction was relatively low in molybdenum, containing only 0·01 p.p.m. of this element, whilst the greater part of the molybdenum-containing impurities were retained on the 24 mesh sieve (10). The sand was further purified by continuously circulating 250 lb. batches with steam for about five hours in the presence of 12 per cent. hydrochloric acid (A.R. grade) and 0·5 per cent. oxalic acid in an automatic apparatus which has been described in detail elsewhere (10). Each batch was treated twice and leached free of acid before use with a purified basal nutrient solution until the pH was unchanged on standing overnight.

Water was obtained by a single redistillation, in continuous-acting pyrex glass stills, of water obtained from tinned copper stills. The molybdenum content of the water prior to redistillation was 0·0003 p.p.m.

The method used for the purification of the basal nutrient reagents was based on the procedure developed by Scott and Mitchell (17) for the concentration of trace elements for spectrographic analysis by co-precipitation with 8-hydroxyquinoline and iron (or aluminium). The basal stock solutions of nutrient reagents consisted of 2 molar calcium and potassium nitrates, molar sodium dihydrogen phosphate and 1·5 molar magnesium sulphate. These were prepared and purified as follows: Calcium nitrate was prepared from glass-distilled nitric acid and re-precipitated calcium carbonate while the others were available either as "batch analysed" A.R. grade or as normal recrystallized grade; these were first recrystallized twice, with the addition of purified alcohol to greatly enhance the yields (10). Solutions of each of the four reagents were prepared such that the requisite weight for 2 litres of solution was dissolved in about 1,700 ml. of water. Redistilled hydrochloric acid was added to each to adjust the pH to 1·8 and ferric chloride then added equivalent to 40 mg. of iron, followed by the equivalent weight of 8-hydroxyquinoline. Normal potassium hydroxide solution (A.R. grade) was added with continual stirring until the solution had a pH of 5·0 as determined by a glass electrode. The solution was left to stand overnight, filtered on a Buchner funnel, and the volume made up with glass-distilled water to 2 litres in acid-cleaned pyrex glass bottles. Ferric citrate was used in the nutrient solution to supply iron. This was prepared by first extracting an acidified solution of ferric chloride (containing 47 grm. per litre of iron) with toluene 3 : 4 dithiol in amyl acetate to remove molybdenum (22) followed by neutralization with a glass-distilled ammonia solution in the presence of about 20 mg. of 8-hydroxyquinoline until the ferric complex was precipitated. The solution was filtered and the remainder of the ferric ions were precipitated with more ammonia; the ferric hydroxide was converted to the citrate by boiling in a solution of recrystallized A.R. grade citric acid.

The usual micro nutrient constituents, boron as boric acid, and manganese, copper and zinc as sulphates, were supplied from a separate micro nutrient solution; a second micro nutrient solution was prepared in which molybdenum was also included as ammonium molybdate. The same principal stock solutions were used to prepare both the complete and the "molybdenum omitted" nutrient solutions by dilution with glass-distilled water to produce the following composition:

Reagent.	*grm. in 20 litres.*
$Ca(NO_3)_2$	10·92
KNO_3	6·66
$NaH_2PO_4.2H_2O$	4·16
$MgSO_4.7H_2O$	7·36
$Fe(C_6H_5O_8)$	0·49

Approximate concentrations as milli-equivalents per litre were: Ca^{++} 6·67; K^+ 3·33; Na^+ 1·33; Mg^{++} 3·0; PO_4''' 4; NO_3' 10; SO_4'' 3; Fe^{+++} 0·3; Mn^{++} 0·02; Cu^{++} 0·002; Zn^{++} 0·002; BO_3''' 0·01; pH 5·5-6·0 The molybdenum content of the complete nutrient was 0·015 p.p.m. Mo or 0·0002 milli mol.

Seeds of savoy cabbage, cauliflower (Majestic) and tomato (Market King) were germinated on glass wool with purified nutrient solution. The seedlings were transferred to the final sand cultures when the first true leaf was beginning to expand. Three or four seedlings were transplanted into each beaker on June 27th, 1947, and thinned two weeks later to one or two plants. Mustard seeds were sown directly in the experimental beakers.

Results.

Tomato, cauliflower and savoy cabbage:—Pronounced visual symptoms appeared rapidly in the cultures receiving the " molybdenum omitted " treatment. The symptoms observed in the tomato confirmed those described by Arnon and Stout (3) and were visible within two to three weeks after transplanting the seedlings. The first symptoms appeared in the older leaves. They showed yellow-green mottling diffusely between the main veins and had upcurled margins ; necrosis and shrivelling of leaf tips occurred beginning in the top leaflet and later spreading to all leaflets. The old leaves ultimately became completely withered and had a dry, pale brown, papery appearance ; the petioles tended to point in a downward direction. It was noteworthy that in tomato (and also in the Brassicae) the cotyledons remained bluish-green in colour for several weeks, although marked chlorotic symptoms had developed in the middle and older leaves. Although these symptoms progressed up the plant, the shoot and growing point continued to elongate slowly. The upper leaves were pale yellow green and showed mottling and scorching. The flower trusses withered and failed to set any fruit in most plants or only single small fruits in others. Typical effects are shown in Plates I and II.

The symptoms in savoy cabbage and cauliflower began as marginal and interveinal pale yellow-green mottling of the older leaves and the foliage had a " water-soaked " appearance suggestive of material " cleared " in glycerine. The mottling was followed rapidly by incurling and withering of the margins and tips of the leaves, and the older leaves finally shrivelled and dropped off. These symtoms, as shown in Plates III, IV and V, appeared in three weeks, but after this signs of gradual recovery were noted about six weeks after transplanting, particularly in the savoy cabbage.

This recovery permitted the plants to grow to a more adult stage when the following effects developed. At ten weeks, one cauliflower plant which had partially recovered in vigour developed foliage in which the lateral development of the lamina was partially suppressed ; the resultant elongated leaves had a narrow, irregular, wavy outline, and extensive grey-brown withered areas developed between the veins. Younger leaves developed with defective laminae and some showed an extreme condition with only smooth, bare petioles. Death or rather abortion of the growing point then occurred, leaving short rudimentary stumps with brown tips. These bare petioles and many of the defective leaves continued to elongate, rapidly at first, as may be judged by a comparison of Plates VI and VII (shown on the same scale).

In the remaining plants the middle leaves also developed abnormally. In these the leaf tips turned brown and withered, and the petioles were stunted and almost devoid of lamina except for the stipule-like portions around the leaf base. These petioles also continued to elongate, but less rapidly than in the plant already described. Death of the growing point also occurred in these plants and in one, this effect appeared suddenly (as far as could be judged) when the youngest almost rudimentary leaves " deliquesced " overnight to a brown gelatinous mass which, after drying, revealed a smooth, almost formless stump in the centre of the plant. In spite of the death and collapse of the youngest leaves the older leaves still remained turgid, although they were pale green and diffusely mottled. These leaves were markedly downcurled at the tips. The stages described here are shown in Plate VIII.

The savoy cabbage was less spectacular in the later stages of the deficiency. The early mottling persisted in the older leaves and the plants failed to " heart ".

The petioles had a marked tendency to point in a downward direction and the habit remained " open ". The middle leaves showed a discontinuous brown marginal necrosis that resulted in an irregular outline and in some plants led to somewhat narrow ragged leaves ; the leaf tip turned brown and expansion was checked. Death of the growing point had not occurred by the end of October, but in one plant axillary sprouts developed and the young leaves of these, and also the growing points of several, turned brown and died.*

Mustard.—Seeds of white mustard were sown later in the season, on September 12th, 1947, in sand in which oat plants (Spring Oat S.187) had been grown previously without producing the characteristic symptoms noted by Piper (15) using the Algerian oat, although yields were reduced by lack of molybdenum as shown in Table I.

The mustard, however, showed well defined visual symptoms that were analogous to the early symptoms in tomato and the Brassicae. The foliage was pale green or yellow-green and the middle expanded leaves curled forward at the margins, while older leaves showed buff coloured marginal scorching. These effects are shown in Plate IX.

Molybdenum deficiency and nitrate status. The reports in the literature that molybdenum is particularly important to nitrogen-fixing organisms (7), (8), (11), and that utilization by *Aspergillus niger* of ammonium nitrogen is more efficient than of nitrate nitrogen in the absence of molybdenum (18), (19), led to examination of the nitrate status of tissue extracts (14) of freshly sampled leaves of the plants. The results are shown in Table II, where it will be seen that marked accumulation of nitrate occurred in leaf (petiole) tissues of most of the plants starved of molybdenum, and that where clay pots were used for the molybdenum-omitted treatment, intermediate values were obtained. Savoy cabbage, however, did not show such variation in nitrate status and for this no explanation can be given at present.

Portions of epidermal tissue from the leaves of control and molybdenum-deficient plants were examined in a series of glucose solutions of known concentrations. Approximate osmotic pressure values were deduced from the concentration of glucose that produced slight plasmolysis in 50 per cent. of the stomatal guard cells. The results are also given in Table II. There was an increased osmotic pressure in the leaves of those plants which also showed accumulation of nitrates.

Leaf injection and painting experiments with ammonium molybdate solutions. In order to obtain further evidence that the effects described were due to molybdenum deficiency some leaf painting and injection experiments were made. A pair of tomato plants growing in the same beaker was selected four weeks after transplanting

* In another part of these experiments not described in detail here, 10-inch clay pots painted with three coats of a pure bitumen solution were used in place of the pyrex glass beakers, but all other details were similar. Equally severe early symptoms appeared in all the crops mentioned, but recovery occurred relatively rapidly and subsequent growth appeared normal ; tomato plants fruited, and cauliflowers produced fair flower heads. These effects demonstrated the fact that these containers were apparently able to supply sufficient molybdenum for growth. In earlier (unpublished) experiments made by one of us (E.J.H.) using a less effective procedure with such clay pots, mottling resembling that seen in the older leaves of savoy cabbage and cauliflower was observed. The cauliflowers produced " cull " heads with rapidly extending pedicels which later wilted and collapsed in a manner similar to that produced by lack of calcium. The flowers aborted and scarcely any seed was set, although some " pods " developed, but the seeds remained green or pale brown, and finally shrivelled. (See Plate X.)

TABLE I.

Yields in grm. of oat straw and grain for control and molybdenum-deficient cultures harvested on September 2nd, 1947, after 12 weeks' growth.

	Straw (10 plants).	Grain (10 plants).
Pyrex glass beaker with molybdenum	49·5	10·9
Pyrex glass beaker without molybdenum	22·7	3·85
Clay pots with molybdenum ..	55·0	8·4
Clay pots without molybdenum ..	15·9	2·2

TABLE II.

Nitrate content as p.p.m. in fresh leaf tissues and approximate osmotic pressure in atmospheres of stomatal guard cells of control and molybdenum-deficient plants.

	CROP.									
	Tomato.			Cauliflower.			Mustard.		Savoy cabbage.	
Date sampled.	−Mo in glass beakers.	−Mo in clay pots.	+Mo control.	−Mo in glass beakers.	−Mo in clay pots.	+Mo control.	−Mo in glass beakers.	+Mo in glass beakers.	−Mo in glass beakers.	+Mo control.
Nitrate content. 11.9.47 ..	240	50	NIL	400	150	30	—	—	165	205
6.10.47 ..	170	—	5	100	—	45	100	15	60	70
Osmotic pressure. 12-14.11.47	2·24	—	0·90	13·44	—	10·08	8·96	3·92	4·48	10·08

when both showed pronounced symptoms; one was injected on July 25th, 1947, by removing the apical leaflet of a lower leaf and inserting the stalk into a light capillary tube drawn out from a small reservoir containing a solution of 10 p.p.m. molybdenum as A.R. grade ammonium molybdate. The stalk fitted closely into the capillary which was kept horizontal and no leak or dripping occurred. In forty-eight hours the mottling had disappeared from all leaves above the injected leaf and the colour changed from pale yellow-green to dark green. Rapid elongation occurred, and all fresh growth was a normal dark green, the leaflets were flat instead of being upcurled at the margins as in the untreated plant and fruiting occurred normally. The heights of the injected and untreated plants were 15 and 18 cm. respectively at the time of the experiment, and twenty-five days later they were 63 and 50 cm. respectively; this represented an overall increase of 50 per cent.

in the growth rate of the injected plant. No recurrence of symptoms appeared in the latter by the end of October, although shoot elongation again slowed down. It was estimated that the total amount of molybdenum injected did not exceed 0·01 mg. In other experiments, made by painting leaf surfaces of tomato and savoy cabbage or by placing a drop of solution on a small incision in a leaf vein of cauliflower plants showing severe symptoms, with a solution containing 100 p.p.m. of molybdenum, there were similar rapid recoveries, but symptoms reappeared in the Brassicae after four to five weeks, following this type of application.

Influence of 8-hydroxyquinoline. Although control cultures receiving molybdenum produced normal plants and were supplied with nutrient solution from the same source as those deficient in molybdenum it was considered desirable to examine whether any of the effects described had resulted from the presence of traces of the 8-hydroxyquinoline added during the purification of the stock nutrient solutions, although it was expected that most of this compound would have been removed by the precipitation procedure. In order to test this point an additional series of cultures was set up in which 8-hydroxyquinoline was present in a much greater concentration in the complete nutrient, namely 3·6 p.p.m., this being the concentration that would have resulted had none of the 8-hydroxyquinoline been removed in the purification process. The plants grew normally with this solution and showed no toxic effects, and it was concluded that the results observed were not due to the action of traces of 8-hydroxyquinoline remaining in the nutrient solution after purification.

DISCUSSION.

Although most of the observations described here are the result of a single experiment, certain points seem sufficiently outstanding to merit discussion. The responses to injection, and other methods of leaf application of molybdenum-containing solutions, afforded evidence that the effects produced were due to omission of molybdenum, whilst extraneous interactions from 8-hydroxyquinoline appeared to be absent.

The morphological distribution of deficiency symptoms in tomato suggested that for this plant, trace supplies of molybdenum could readily be translocated from older leaves to younger ones for re-utilization in the growing shoot. The cauliflower and savoy cabbage plants similarly showed effects in the older leaves, particularly in the earlier stages of the growth. As the deficiency developed, however, (subsequent to a temporary recovery), the most severe symptoms appeared in the younger parts, and ultimately led to death of the growing point. Symptoms observed in lettuce by Warington (21) were mainly in the "middle" leaves, a condition that might be analogous to effects shown more particularly by savoy cabbage in the present experiment. The distribution of symptoms in mustard showed that the middle and older leaves were most affected, but the later sowing and slower growth conditions do not at present justify greater emphasis on the particular response of this crop. The symptoms shown in the cruciferous species therefore suggests that the re-distribution of molybdenum in deficient plants may occur less readily than in tomato and that it may be insufficient to prevent death of the growing point. Moreover, the importance of molybdenum may vary somewhat during the life history of the plant as is suggested by the effects of molybdenum deficiency on flowering

and seed formation in cauliflower, on fruit formation in tomato, and on yield of grain in oat. Bertrand (4), (5), has found that the cruciferous species are relatively rich in molybdenum and that the seeds in particular accumulate this element. The mobility of limited amounts of molybdenum in the deficient plants may also vary with age, or stage of development in some species, and the long period during which the cotyledons of deficient seedlings remained green may be significant in this respect. The elucidation of these points must await further experiments.

Two other points call for comment at this stage. The capacity for continued extension shown by many practically bare petioles after death of the main growing point had occurred was particularly noteworthy. The reason for the elongation is not known. It was also observed that old leaves of molybdenum-deficient savoy cabbage plants seemed to be unusually large as compared with those of the control plants, but measurements were not made. This capacity for continued expansion of relatively mature organs is of interest. The rapid elongation of bare petioles and the development of narrow wavy laminae of middle leaves of one of the cauliflower plants shown in Plate XI, appeared identical with the field condition known as " Whiptail " (see Plate XII) which has already been reported to respond to dressings of molybdenum (9), (13). Unpublished observations by one of us (E.J.H.) show that death, or abortion of the growing point, is also frequently associated with plants affected with " Whiptail ". The narrowing of middle leaves of savoy cabbage plants was again suggestive of the same type of response.

The difference in nitrate contents of the leaf extracts from molybdenum-deficient and normal plants was consistent with the trend in the osmotic pressures of the stomatal guard cells and supports the view that molybdenum is involved in the nitrogen metabolism of higher plants. It is possible that some step in the reduction of nitrates is inhibited in the absence of molybdenum. The work of Bortels (7), (8) and of Horner *et al.* (11) with nitrogen-fixing organisms, also implies a relation between nitrogen metabolism and molybdenum, as does the differential response of *Aspergillus niger* to ammonium and nitrate forms of nitrogen when molybdenum is withheld from the nutrient (18), (19). The results obtained on nitrogen fixation in lucerne by Jensen and Betty (12) and the fact that symptoms of molybdenum shortage in leguminous pasture plants (1) may resemble nitrogen deficiency is additional support for this view.

SUMMARY.

1. A method is described for the study of molybdenum deficiency in plants grown in sand culture and a new method is outlined for the elimination of molybdenum from nutrient reagents.

2. Severe symptoms caused by lack of molybdenum were observed in tomato, cauliflower, savoy cabbage and mustard, and yields of oat straw and grain were also greatly reduced.

3. Injection methods to supply small amounts of molybdenum produced rapid recoveries in deficient plants.

4. There was a marked accumulation of nitrate in the leaf (petiole) tissues and increased osmotic pressures in the stomatal guard cells of cauliflower, mustard and tomato plants from which molybdenum was withheld.

5. Certain symptoms shown by molybdenum-deficient cauliflower plants appeared to be identical with the condition known as " Whiptail ".

6. The distribution of deficiency effects in the tomato and in some cruciferous species is discussed in relation to their life histories and the mobility of molybdenum in these plants.

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PLATE I.

Tomato plants. Early stages of molybdenum deficiency.

PLATE II.

Tomato leaves.

Centre : Leaf from control plant.
Sides : Leaves from "molybdenum omitted" plants. Note chlorotic mottling, marginal curling, and necrosis, commencing from tips of leaflets.

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PLATE III.
Cauliflower seedlings, molybdenum omitted. Interveinal mottling of leaves and withering of leaf tips. Cotyledons remain dark blue-green colour.

PLATE IV.
Savoy cabbage seedlings, molybdenum omitted. Mottling of leaves and withering of leaf tips.

PLATE V.

Savoy cabbage seedlings, molybdenum omitted. Forward curling of leaf margins, and interveinal mottling.

PLATE VI.

(Reduced to 7/35 natural size)

Cauliflower plant, molybdenum omitted. Photographed on 3.10.47.

PLATE VII.

(Reduced to 10/35 natural size)

As Plate VI, but photographed three weeks later on 24.10.47. Note growth of bare midrib.

PLATE VIII.
Cauliflower plant, molybdenum omitted. Death of growing point and tips of young leaves, and loss of lamina.

PLATE IX.
Mustard Leaves.
Left and right : Control. *Centre :* Molybdenum omitted.

PLATE X.
Cauliflower.
Molybdenum omitted. Abortion of flower buds, wilt of pedicels.

PLATE XI.

Cauliflower plant, molybdenum omitted. Elongated narrow wavy laminae, bare petioles and death of growing point as in " Whiptail " specimens.

PLATE XII.

Broccoli plant (field specimen) showing acute " Whiptail ".

REVIEWS

THE GRAFTER'S HANDBOOK. By R. J. GARNER. (London: Faber & Faber. 1947. Pp. 223. Price 15s.)

In a life of almost perpetual motion Mr. Garner, at his work at East Malling, may be seen running, walking, poised precariously in trees and on the topmost rungs of ladders, or curved in the contorted postures inseparable from the practice of his craft, but seldom in repose. That he has found time to sit long enough to incubate this, his long-planned project, is a matter of congratulation for the author and his readers. For, if we mistake not, Mr. Garner has hatched a very notable chick. It can be said with truth that no previous publication has dealt so comprehensively with the art of grafting and all that pertains thereto; for, as the student will soon discover, there is a deal more "to it" than a mere application of scion to stock. The style is brisk and terse, every sentence at once conveys its meaning (high praise for scientific literature)—and the author says it only once. The highlights of the book are, of course, the lucid descriptions of innumerable grafting procedures, each operation admirably illustrated by one or more of the author's own drawings. But there is much to be learned before the student can take his grafting tools in hand. Thus, the preparatory chapters are devoted to a brief study of plant structure and its bearing on the union of stock and scion, to the mysteries of incompatibility and the workings of the cambium. The collection and treatment of scion wood of various kinds is discussed. Scions need stocks, and the many methods of raising these (especially for fruit trees) are well explained. In a Chapter on tools and accessories no less than twelve types of knife are described and illustrated, though it is not suggested that the grafter will need them all. Some are Continental or American types, included for comparison. Chisels, saws and secateurs are noticed. Useful recipes are given for various graft seals and waxes, and a portable wax heater is described and illustrated. Then in Chapter 6 come the grafting methods, most handsomely dealt with, as already indicated. The concluding Chapters show how what has been learned may be applied in the field to the raising of trees in nurseries and to the grafting of old-established trees for conversion or repair. There is a list of over one hundred references and two appendices. Of these last, the first gives a list of pears which are better double worked when on quince rootstock, the suggested intermediate being Beurré Hardy; the other tabulates compatible rootstocks for some of the principal plum varieties. For the reason that it embodies the accumulated knowledge of years of close research and practical work, carried out by an enthusiast who is also a craftsman, and in conditions exceptionally propitious for such studies, this book will fill a gap in horticultural literature from which it is not likely to be displaced.

G. St.C. F.

LAND CLASSIFICATION FOR LAND-USE PLANNING. By G. V. JACKS. Imperial Bureau of Soil Science, Technical Communication No. 43. Pp. 90. 1946. Price 4s. net.

This work, coming at a time when "planning" of all kinds is so very much the vogue, fills a real need. So many systems of classification of land for different purposes have been proposed and the literature on the subject is so widely dispersed

that a critical review of this kind, collating and correlating the relevant material, will prove of great value to anyone concerned in the development of land areas. Inevitably, the subject must draw upon a wide field ; soil science, agriculture, forestry, ecology, economics, regional geography and sociology are all intimately concerned in any system of land classification, and few indeed are those with sufficient knowledge in all these spheres properly to integrate their respective contributions. Dr. G. V. Jacks, as Director of the Imperial Bureau of Soil Science, is unsurpassed in his qualifications to appraise the published matter and place it in correct perspective.

In the sense used in this work the term land classification " relates to the grouping of lands according to their suitability for producing plants of economic importance ". Dr. Jacks stresses the importance of two fundamental points in any system of land classification for planning purposes, first, that the classification must, in the nature of things, be related to the objective of the plan ; this is the chief cause of the diversity of criteria which have been used. Secondly, any land-use plan must normally be based on the conservation of soil fertility. Exploitation of the land for the benefit of the occupiers, resulting, as it must, in the draining of its capital resources, can have no place in a plan designed for the permanent betterment of the people dependent thereon.

After a review of the bases of classification systems the author discusses the types of classification which have been proposed, first those based on physical and chemical soil factors and then those devised according to use capabilities. Of particular interest at the present time is a short section on the estimation of the capacity of different regions to support a population.

The chapter on the use of natural vegetation as an indicator of land quality sweeps up and presents in coherent form the data of many ecologists in all parts of the world, and is of great value in indicating sources of original data.

Most of the remainder of the bulletin is a detailed review of the different systems of land classification which have been proposed by workers in all the chief countries of the world, and a consideration of the various attempts to evolve " productivity ratings " of land in terms of the experience of users. The German systems of allotting a weighted scale of points to the different factors concerned in productivity receives special attention and are considered of sufficient value to justify the reproduction of the official rating tables for arable and grassland.

In an appendix the author reproduces an outline U.S. soil-survey report which will be of interest to the student of the subject in indicating the great variety of factors to be considered in assessing the agricultural value of any region.

CHEMICAL COMPOSITION OF PLANTS AS AN INDEX OF THEIR NUTRITIONAL STATUS. By D. W. GOODALL and F. G. GREGORY. Technical Communication No. 17, Imperial Bureau of Horticulture and Plantation Crops. (East Malling: Imp. Bur. Hort. and Plantation Crops. 1947. Pp. 167. Price 9s. net.)

In Professor Blackman's foreword to this latest of the valuable series of Technical Communications from the Imperial Bureau of Horticulture, he states that " timeliness and promise " have been claimed as proper tests of a research investigation. Of the first of these requirements there can be no doubt of the present volume's fulfilment. The diagnosis and quantitative determination of plant nutritional deficiencies is a problem engaging the attention of many workers in the modern field of crop physiology.

The importance in particular of the "trace" or "minor" elements, and of the disorders and consequent crop losses caused by deficiencies of one or more of them, is very much to the forefront.

Basic to the whole problem is the diagnosis of disorders suspected to be due to, or related to, deficiencies, and a very wide range of different methods of diagnosis have been proposed by different workers. The validity of these methods must, in each case, be referred ultimately to exact chemical analyses, but it is just at this point that the confusion tends to arise, since workers have differed not only in their methods of presentation of results, but in such matters as the choice of plant part for analysis, the selection of a procedure and the quantitative expression of analytical figures.

The authors of this volume have performed a great service in collating all the published work up to 1945, and presenting it in such a form that the validity of the different methods of diagnosis and their relationships to exact chemical analyses may be assessed. Not the least valuable feature of the work is the classification of the aim of a method of diagnosis. The authors' approach is a pragmatic one, as is exemplified by their definition of a deficiency: "a plant is deficient in a certain element if supplying that element to the plant in a suitable form causes an increase in the yield, this effect being specific to the element in question."

The authors first review the methods which have been proposed for diagnosis and the theoretical consideration of the interrelations of nutrient supply, uptake and concentration and final yield, and then pass on to a consideration of the selection of a method for chemical analysis. Methods of expression and interpretation of results are discussed in detail, followed by a valuable review of the factors which may affect the use of plant analysis for diagnosis, such as weather conditions, pests and diseases and varietal differences. Finally, the comparison of plant analyses with field trials, soil analyses and visual symptom diagnosis is critically discussed.

An exhaustive bibliography and an admirable subject-index help to make the publication one of the utmost importance to all workers in the sphere of crop physiology.

GARDEN WORK MONTH BY MONTH. By JAMES WILSON. (Royal Horticultural Society, London. Pp. 47. Price 1s. 6d., post free.)

The substance of this booklet appeared in 1945 as a series of monthly articles in the *Journal of the Royal Horticultural Society*, but there is no doubt that a useful service has been rendered in making the revised articles available in this convenient form. No attempt has been made to produce a complete handbook of gardening, instead the scope has been limited to timely reminders of important jobs that are easily overlooked. There is nevertheless a surprising amount of cultural information packed into the brief space allotted for each month, with advantage to those who forget names of varieties, sowing distances, greenhouse temperatures, spray concentrations, fertilizer amounts, etc. Besides being divided into sections month by month, the information for each month is arranged under five headings, the Flower Garden, the Fruit Garden, the Vegetable Garden, Unheated Greenhouses and Frames, and Continuous Cloches. The whole subject of artificial heat is omitted. Mr. Wilson's wide experience of gardens under many conditions as Garden Inspector of the R.H.S. well qualifies him to pick out for mention the most important operations; this fact, combined with the convenient arrangement of the matter, make this booklet a valuable addition to every gardener's reference library. H.B.S.M.

THE FRUIT YEAR BOOK, 1947. (Royal Horticultural Society. Pp. 114. Price 8s. 6d. net.)

"The Fruit Year Book should serve as a common ground wherein private growers can exchange their experiences and methods of technique with those of the professional grower and the scientific investigator." These words of Dr. R. G. Hatton's in the foreword to the Fruit Year Book for 1947 give an ideal which has certainly been reached in the first number. Notes and observations on practical fruit growing by amateur and professional gardeners find place alongside the articles of their scientific friends from the research stations. It is pleasing to find that overseas horticulturists are also represented; H. B. Tukey's account of the growth, decline and now again the rise of "Backyard Gardening in the Eastern U.S.A." has a parallel in what has happened in this country, and we also are now seeing a renewal of interest.

A survey of the many problems facing an amateur who is laying out a fruit garden would be of little use on its own, but it is followed by the concrete suggestions of varieties and plans by such acknowledged experts as J. M. S. Potter and J. W. Bultitude. The established garden is not neglected, although perhaps the subjects dealt with are of a more specialist nature. Peach culture occupies quite a large portion of the book, there being four papers describing the growing of peaches on walls, as bushes and under glass. In spite of this it is likely that there are still other proved and satisfactory methods and that the subject has by no means been exhausted.

There is a more gloomy side however; a short description of the decrepit state of fruit trees in many gardens to-day is unfortunately all too true. Probably the soft fruit is in the worst plight, due to a gradual degeneration of stocks through mixture of varieties and the virus troubles. Some of these troubles are described and well illustrated by H. B. S. Montgomery, who, at the same time, stresses the importance of buying certified plants where available. Short accounts are given of some of the Fruit Group's excursions to places of particular interest and also to three gardens belonging to members of the Group. Space prevents comment on each individual paper in this varied and interesting book; suffice it to say that the subject of fruit growing in this country is brought to a fitting conclusion by an account by H. H. Crane on growing apples for exhibition, and an article by Miss B. A. Crang on fruit preservation.

The Fruit Group deserves congratulations on this their first Year Book, which should assist amateurs with many of their problems. Besides being tastefully bound, it contains numerous photographic plates, line drawings and a comprehensive index. It seems certain that the book will be in great demand.

L. R. E. Martin.

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